SYNTHESIS OF SUBSTRATE ANALOGUES FOR THE METHYLERYTHRITOL PHOSPHATE PATHWAY

by

Sergiy G. Krasutsky

A dissertation submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Chemistry

The University of Utah

August 2011

The University of Utah Graduate School

STATEMENT OF DISSERTATION APPROVAL

The dissertation of **Sergiy G. Krasutsky**

has been approved by the following supervisory committee members:

C. Dale Poulter	, Chair	**01/25/2008** Date Approved
Cynthia J. Burrows	, Member	**01/25/2008** Date Approved
Peter F. Flynn	, Member	**01/25/2008** Date Approved
Eric W. Schmidt	, Member	**01/25/2008** Date Approved
Peter A. Beal	, Member	**09/09/2010** **Date Approved**

and by **Henry S. White** , Chair of the Department of **Chemistry**

and by Charles A. Wight, Dean of The Graduate School.

ABSTRACT

Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), precursors for the biosynthesis of terpenoids, can be formed either by the mevalonate or methylerythritol phosphate pathway. The methylerythritol phosphate (MEP) pathway is not present in humans, therefore the enzymes of the pathway are potential targets for the development of novel antibiotic agents. Five steps, catalyzed by the ispD-H proteins, are required for the enzymatic conversion of MEP to IPP and DMAPP. Analogues for MEP, aminomethylerythritol phosphate (NMEP), methylerythritol thiophosphate (MESP), and thiomethylerythritol phosphate (SMEP) were synthesized and their enzymatic activity for conversion to the cyclic intermediates by the consecutive action of three proteins, ispD, ispE, and ispF, is examined. The last step of the pathway, catalyzed by the ispH, requires elimination of the hydroxyl group from hydroxydimethylallyl diphosphate (HDMAPP), the immediate precursor of IPP and DMAPP. Analogues of HDMAPP, aminodimethylallyl diphosphate (NDMAPP), and thiodimethylallyl diphosphate (SDMAPP) were synthesized as substrates or inhibitors for ispH protein.

TABLE OF CONTENTS

LIST OF SCHEMES

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AcOH	acetic acid
ATP	adenosine triphosphate
BSA	bovine serum albumin
$CDCl_3$	deuterochloroform
CDP-ME	4-diphosphocytidyl-2-*C*-methyl-D-erythritol
MESP	methyl methylerythritol thiophosphate
CH_2Cl_2	methylene chloride
CI	chemical ionization
cMEPP	2-*C*-methyl-D-erythritol-2,4-cyclodiphosphate
CTP	cytidine triphosphate
DMAP	dimethylaminopyridine
DMAPP	dimethylallyl diphosphate
D_2O	deuterium oxide
DX	1-deoxy-D-xylulose
DXP	1-deoxy-D-xylulose 5-phosphate
DXS	1-deoxy-D-xylulose 5-phosphate synthase
FPP	farnesyl diphosphate
GPP	geranyl diphosphate
G2P	glyceraldehyde 2-phosphate

HDMAPP	4-hydroxydimethylallyl diphosphate
HMG-CoA	hydroxy-3-methylglutaryl-coenzyme A
HRMS	high resolution mass spectrometry
Hz	hertz
IC_{50}	inhibition concentration
IPP	isopentenyl diphosphate
i-PrOH	isopropyl alcohol
IPTG	isopropyl-β-D-thiogalactopyranoside
ME	2-C-methyl-D-erythritol
MeOH	methanol
MEP	2-*C*-methyl-D-erythritol 4-phosphate
MESP	methyl methylerythritol thiophosphate
$MgSO_4$	magnesium sulfate
MHz	megahertz
μCi	microcurie
MVA	mevalonate
$NaBH_4$	sodium borohydride
$NADP^+$	oxidized nicotinamide adenine nuclueotide diphosphate
NADPH	reduced nicotinamide adenine nuclueotide diphosphate
Ni-NTA	hexa-histidine binding nickel-charged resin
NDMAPP	aminodimethylallyl diphosphate
NMEP	aminomethyl methylerythritol phosphate
NMR	nuclear magnetic resonance

SDMAPP	thiodimethylallyl diphosphate
SMEP	thiomethyl methylerythritol phosphate
TBAF	tetra-*n*-butylammonium fluoride
TBDMSCl	*tert*-butyldimethylsilylchloride
TBDPSCl	*tert*-butyldiphenylsilylchloride
THF	tetrahydrofuran
TLC	thin layer chromatography

ACKNOWLEDGMENTS

I wish first to express my sincere gratitude to my advisor, Dr. C. Dale Poulter, for his guidance, and support, for being always patient and helpful when he was most needed.

I would like to thank Dr. Joel Walker and Dr. Guillermo Labadie for their assistance and useful discussions throughout my studies.

I am also very thankful to Dr. Burrows, Dr. Flynn, Dr. Schmidt, and Dr. Beal for being members of my thesis committee and their input in my work.

Finally, many thanks go to the Department of Chemistry for the opportunity to work and study here.

CHAPTER 1

INTRODUCTION

Background

Isoprenoid compounds represent one of the most chemically diverse families in nature, with more than 55 000 identified specimens to date.[1] They exhibit a broad range of structural complexity and are involved in a variety of biological processes. These functions include mediation of cell-wall and glycoprotein biosynthesis (dolichol diphosphates), electron transport and redox chemistry (ubiquinones), modification of tRNA (N^6-isopentenyladenine), posttranslational modification of proteins (farnesylated proteins), photooxidative protection (carotenoids), contribution to lipid membrane structure (cholesterol), antitumor compounds (taxol), as antibiotics (terpentecin), and phytoalexins (trichothecin) (**Figure 1.1**).[2]

The structural diversity of isoprenoid compounds is formally derived from the branched C5 skeleton of isoprene. Both dimethylallyl diphosphate (DMAPP) and isopentyl diphosphate (IPP) correspond to the biological equivalents of isoprene. The carbon skeletons of isoprenoid compounds are synthesized by condensing IPP with DMAPP (or DMAPP like allylic diphosphates) or condensing two molecules of DMAPP (or DMAPP like allylic diphosphates). Isoprenoid molecules are constructed from IPP and DMAPP through a series of alkylation reactions mediated by prenyltransferases.

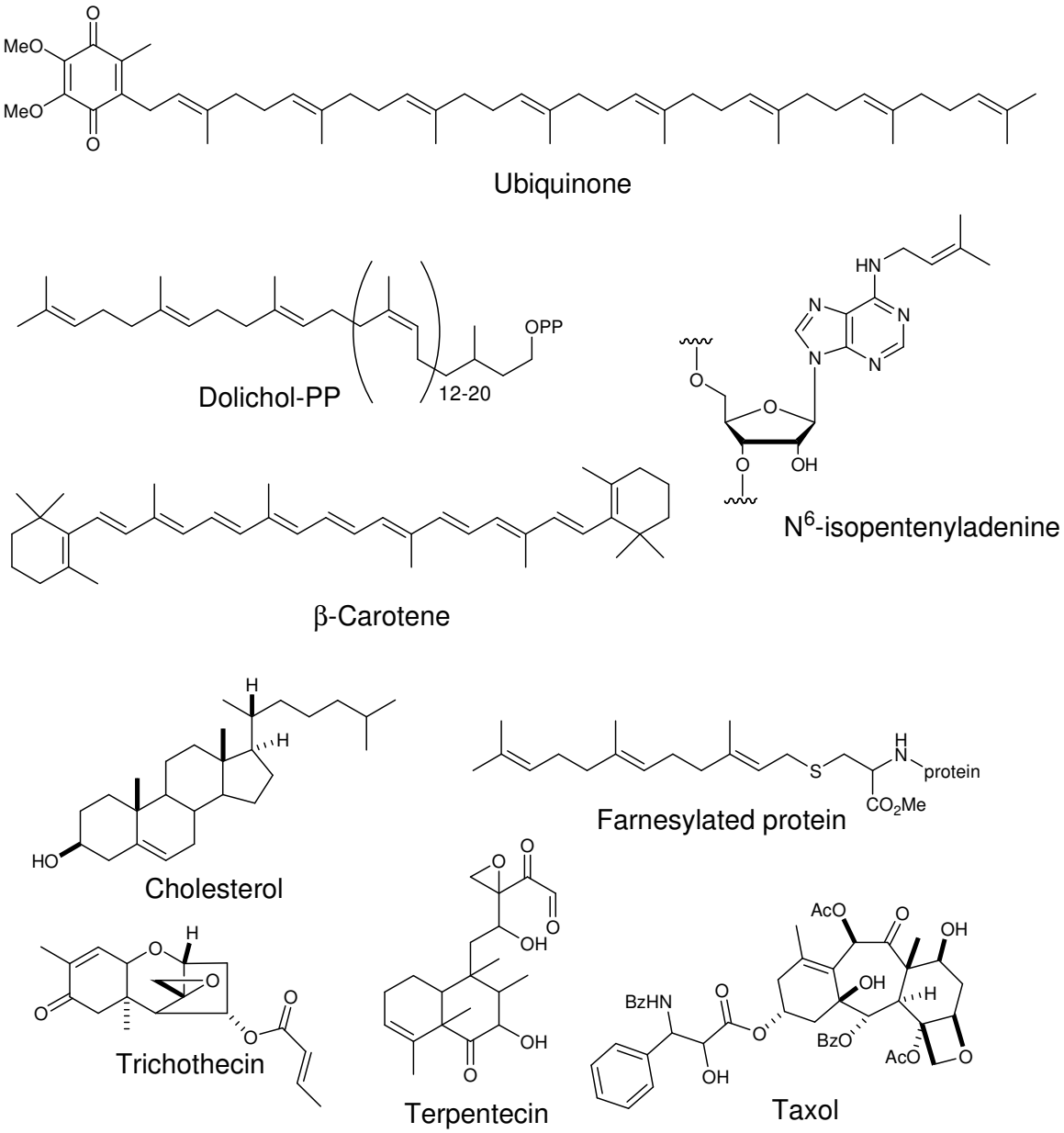


Figure 1.1. Structures of the mentioned isoprenoids.

These alkylations include "*Chain elongation*," 1'-4 coupling between IPP and DMAPP to obtain molecules, like geranyl diphosphate; "*Branching*," 1'-2 connection of two allylic diphosphates, like lavandulol; "*Cyclopropanation*" - c1'-2-3 connection, to form molecules like chrysantamyl diphosphate; *Cyclobutanation*" - c1'-2-3-2' connection. These possible connections are shown in **Figure 1.2**.

The mechanisms for these steps proceed by dissociative electrophilic alkylation. The cations generated from DMAPP (or DMAPP like diphosphates) alkylate the double bond of IPP or DMAPP. Heterolytic cleavage of the bond between the diphosphate moiety and C1 of DMAPP generates an allylic carbocation. The double bond of IPP is alkylated by the allylic cation to generate a tertiary cation. An external base removes the pro-R proton from the IPP unit to form an *E* double bond in GPP (**Figure 1.3**).[3] These mechanisms are shown on **Figure 1.3** and **Figure 1.4**.

The mechanism of cyclopropanation, branching, and cyclobutanation can be represented in a similar manner. Heterolytic cleavage of the bond between the diphosphate group and C1 of DMAPP generates an allylic carbocation. Alkylation of allylic cation by the double bond of DMAPP produces a protonated cyclopropane intermediate (**Figure 1.4**).

The rearrangement of the protonated intermediate gives several final products. First, it can be deprotonated to give chrysantamyl diphosphate. Second, it can open and either be deprotonated to give lavandulol diphosphate or cyclize to a cyclobutyl structure and subsequently deprotonated to give maconelliyl and planococcyl diphosphates (**Figure 1.4**).

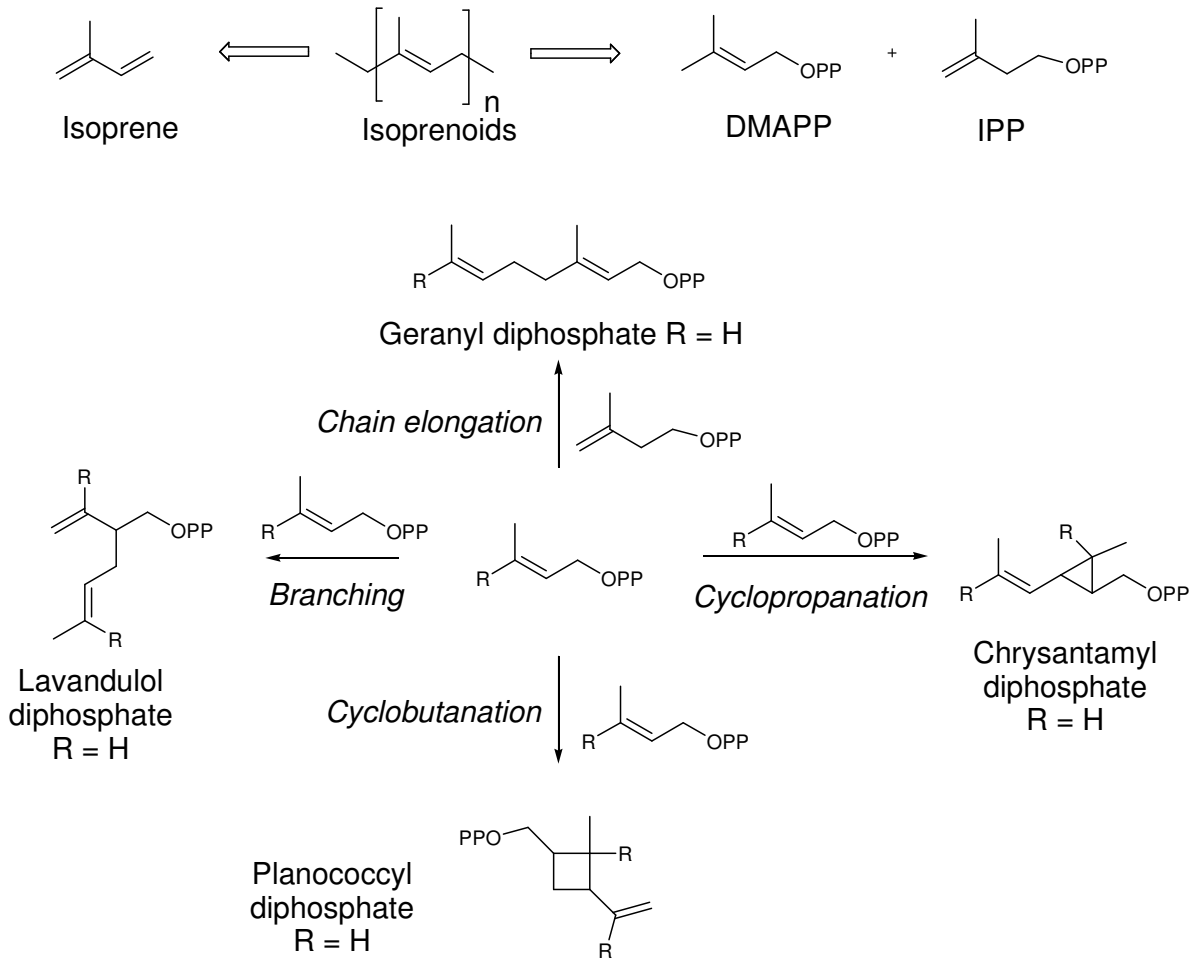


Figure 1.2. Possible connections for isoprenoids.

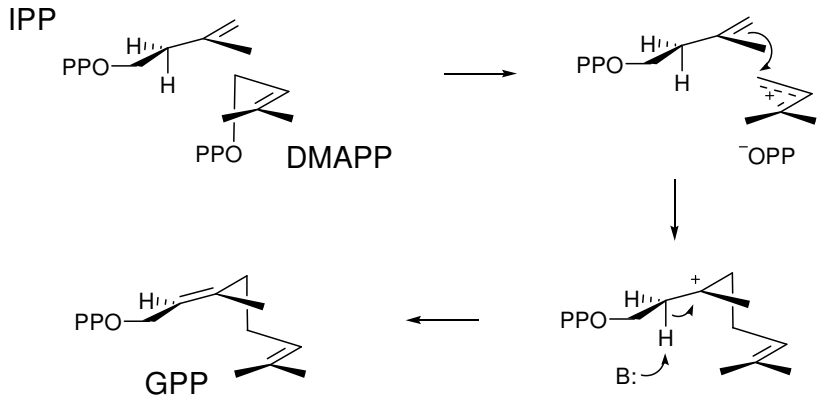


Figure 1.3. Mechanism for chain elongation.

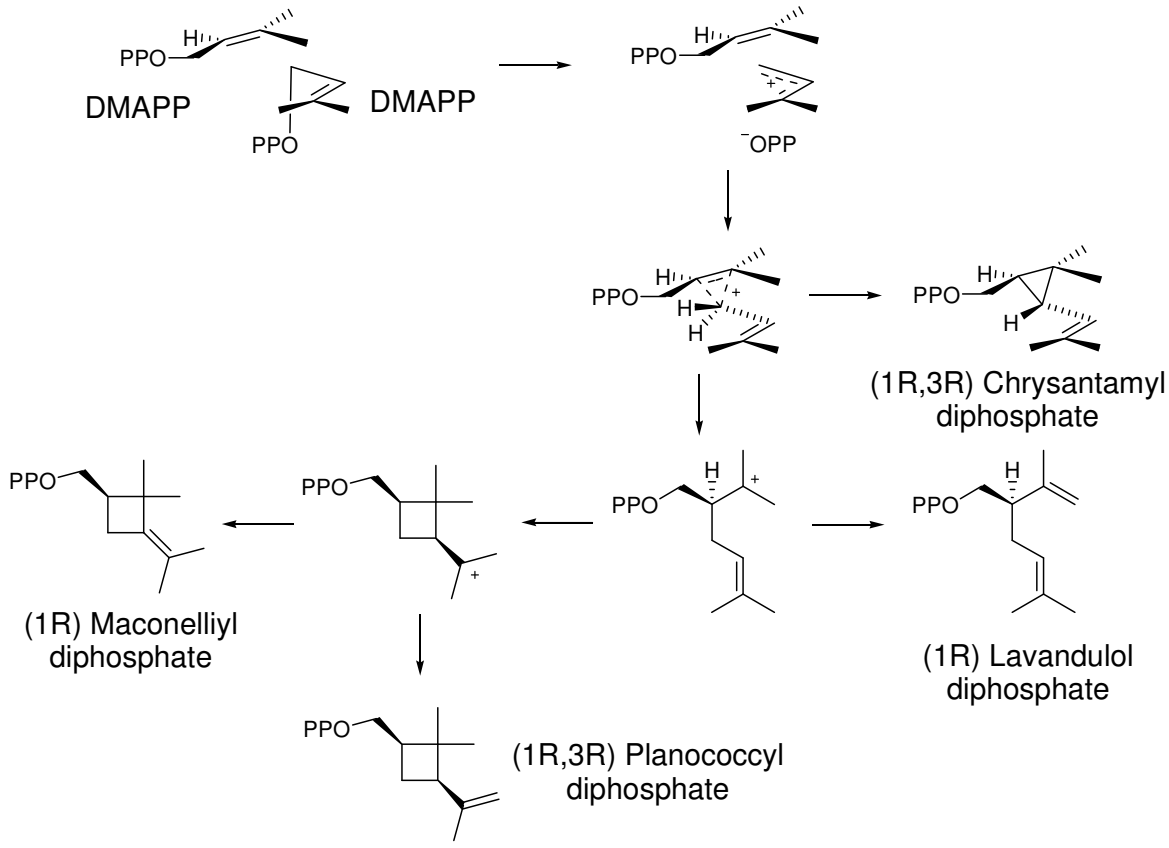


Figure 1.4. Mechanism for cyclopropanation, branching, and cyclobutanation.

The biosynthesis of higher order isoprenoids such as geranyl diphosphate, farnesyl diphosphate, and geranylgeranyl diphosphate arise from chain elongation coupling between IPP and DMAPP (**Figure 1.5**). These compounds are important intermediates for the biosynthesis of other isoprenoids including cyclic compounds. For example, cyclization of geranylgeranyl diphosphate (GGPP) to the bicyclic terpene – *ent* – copallyl diphosphate proceeds by protonation of the double bond followed by a pair of electrophilic cyclizations.[2]

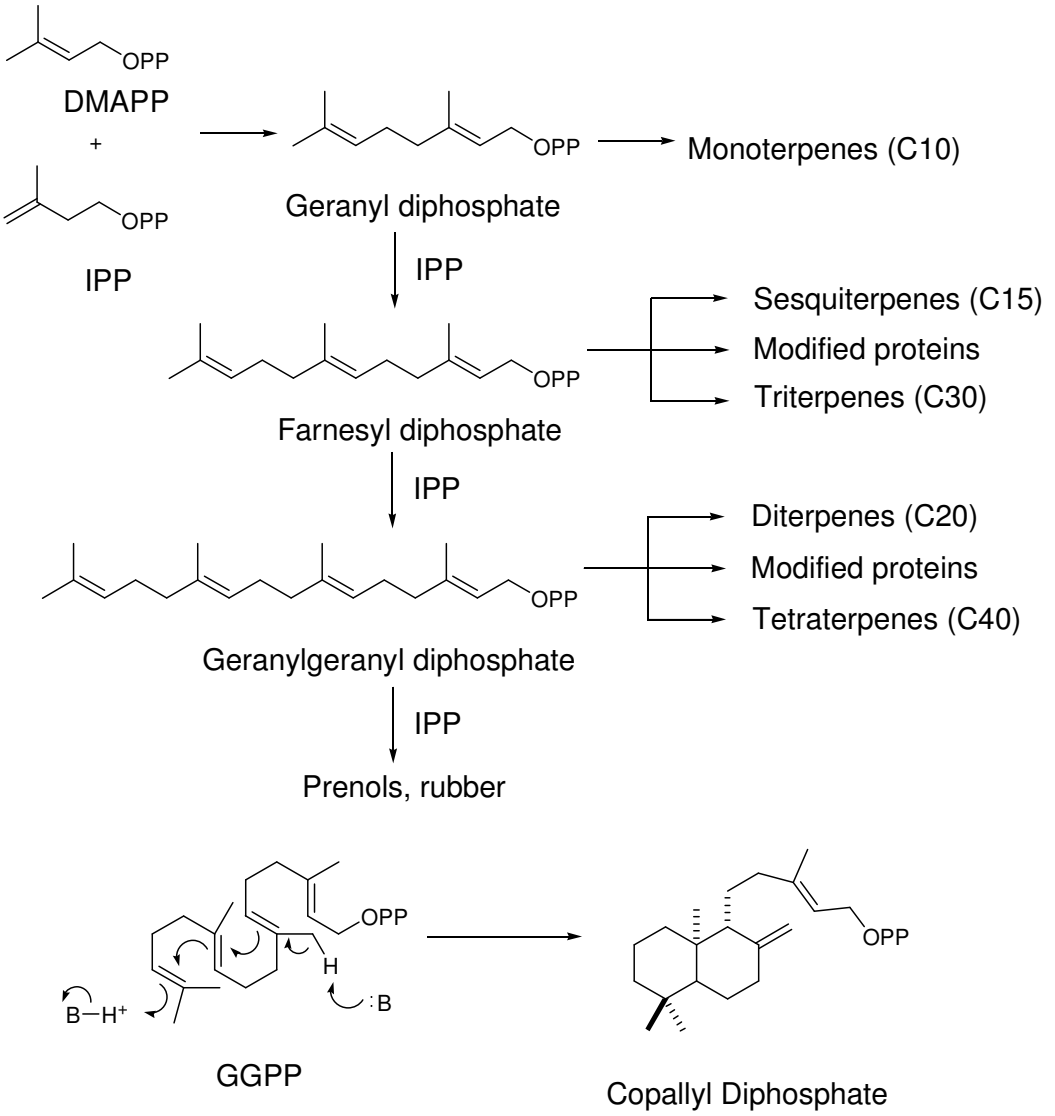


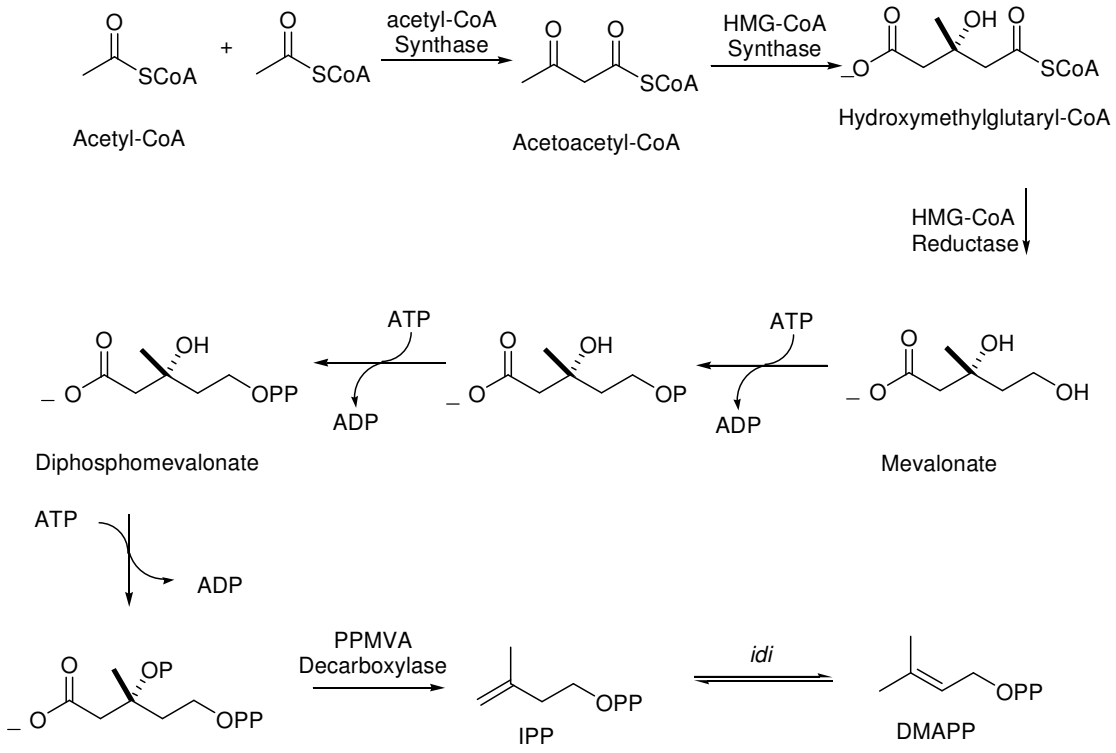
Figure 1.5. Linear expansion of isoprene units, also showing cyclization.

Mevalonate Pathway to IPP and DMAPP

Isoprenoid biosynthesis depends on the synthesis of IPP and DMAPP. The elucidation of the biosynthesis of IPP and DMAPP resulted in the discovery of the mevalonate (MVA) pathway in the 1950s. This work was performed with liver tissues and yeast during studies of cholesterol and ergosterol and biosynthesis was later confirmed for the biosynthesis of plant sterols and triterpenes. As a result, mevalonate was unanimously accepted as the universal isoprenoid precursor in all living organisms, despite some contradictory results obtained when working with chloroplasts and some bacteria.[4]

The MVA pathway begins with a Claisen condensation followed by an aldol condensation to yield acetoacetyl CoA and hydroxymethylglutaryl CoA. A NADPH-dependent reducing enzyme catalyzes the first committed step in the pathway – the reduction of hydroxymethylglutaryl CoA to mevalonate. Three successive phosphorylations followed by decarboxylation finally yield a five-carbon atom skeleton of IPP. IPP isomerase rearranges IPP to DMAPP (**Scheme 1.1**).

It was assumed for several decades that bacteria,[5] plants,[6] and green algae[7] synthesize IPP and DMAPP from acetyl-CoA according to the well-established MVA pathway. However, some discrepancies in the MVA pathway were reported. ^{14}C-labeled mevalonate is poorly incorporated into carotenoids from plant chloroplasts;[8] mevinolin, a potent inhibitor for HMGCoA reductase, had no effect on carotenoid biosynthesis;[9] The incorporation of ^{13}C-labeled and ^{14}C-labeled acetate into prenyl chains in *E.coli* resulted in a radioactive distribution that did not fit the expected pattern for the MVA pathway.[10]



Scheme 1.1. Mevalonate (MVA) pathway.

Early on, these contradictory results did not raise concerns about the general idea of MVA pathway for biosynthesis of IPP and DMAPP. For example, the negative result with mevinolin was attributed to impermeability of the chloroplast membrane.[11]

An alternative pathway was discovered during studies of hopane biosynthesis. Hopanoids are a group of pentacyclic triterpenes, structurally similar to steroids which are synthesized by cyclization of squalene and probably serve a similar physiological role (**Figure 1.6**).[4,12]

Hopanoids are usually present in cells in high concentrations. For example, in *Z. mobilis* they reach a maximum of 30 mg/g (dry weight).[13] Their availability, chemical stability, and ease of isolation made them attractive for biosynthetic studies with ^{13}C labeled acetate as a single carbon and energy source.[14]

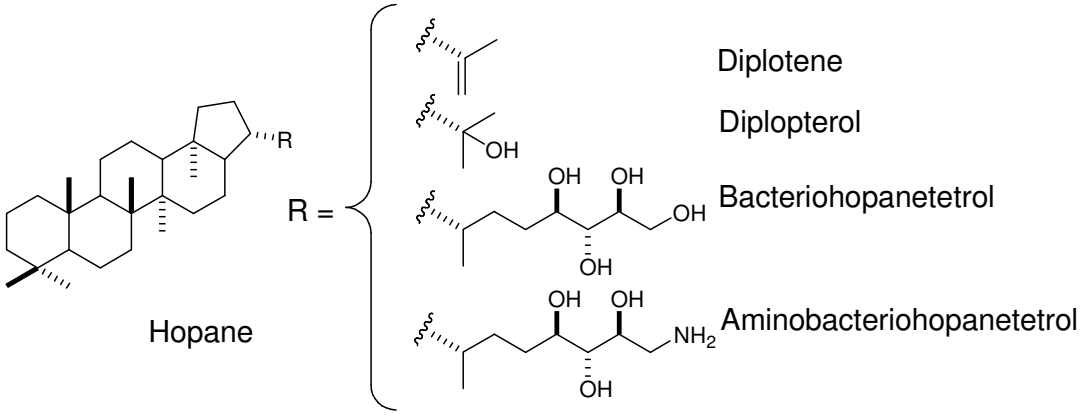


Figure 1.6. Hopanoids.

The interpretation of data showed an unexpected labeling pattern that was inconsistent with the classical acetate-mevalonate pathway (**Figure 1.7**). The existence of two noninterconvertible acetyl CoA pools was hypothesized. However, these data alone were not sufficient to explain the labeling observation. The discovery of the new pathway became clear after the incubation experiments of ^{13}C-labeled glucose, which is metabolized in *Z. mobilius* via Entner-Doudoroff route.[15] After the assignment of all carbon atoms in metabolites derived from IPP and DMAPP, it became evident that there is a novel pathway present, which was named the mevalonate-independent, or later, the methylerythritol phosphate pathway.[16]

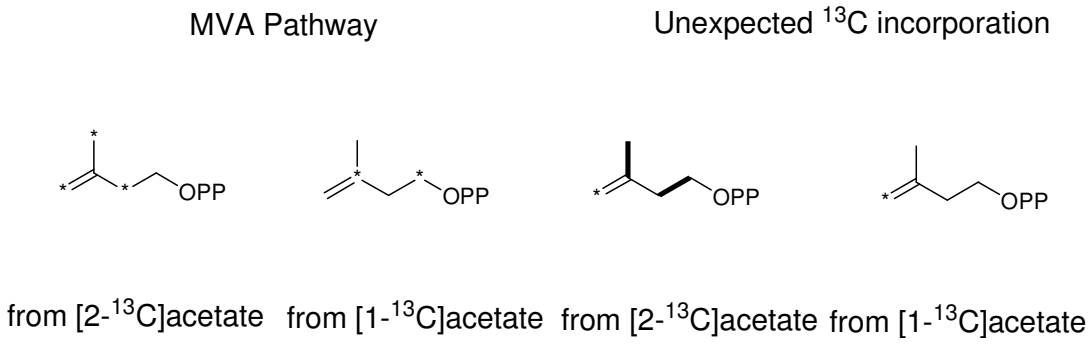
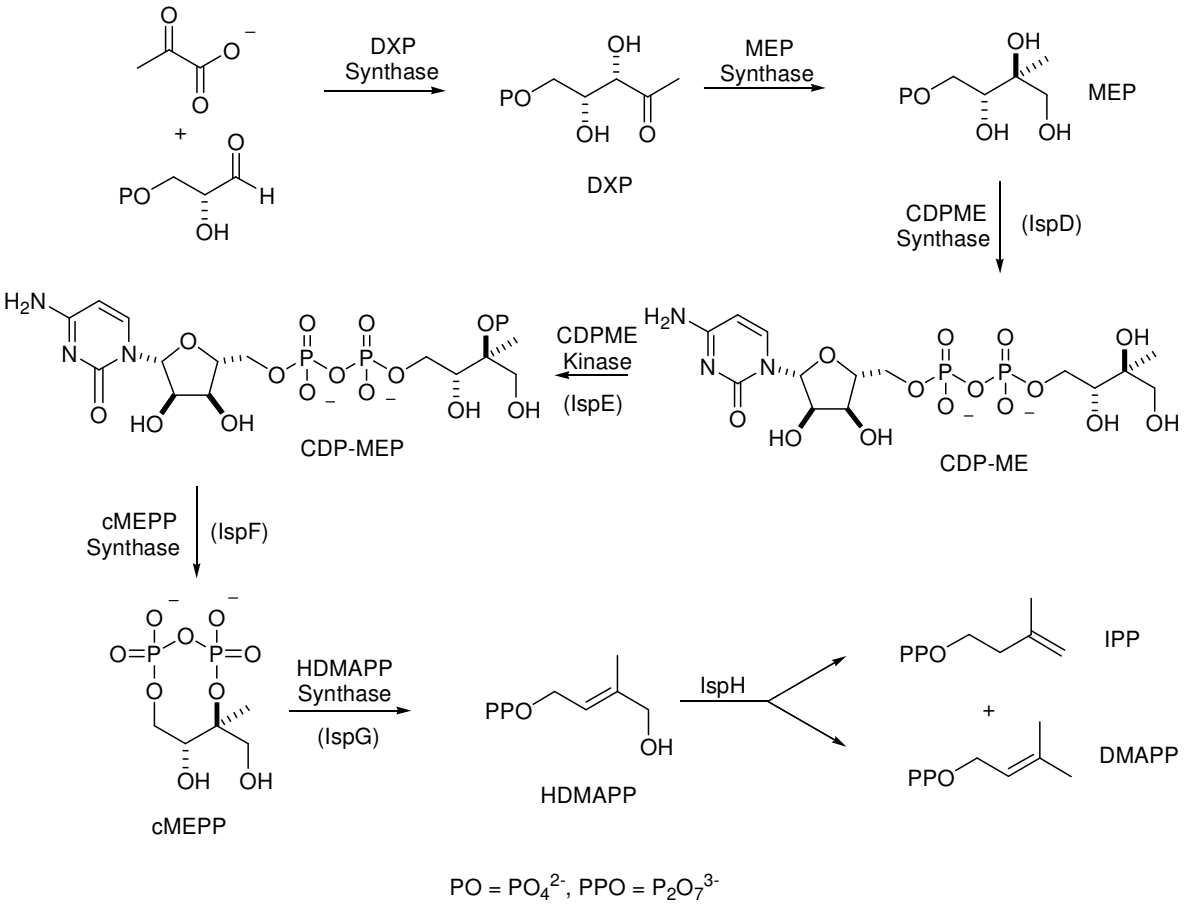


Figure 1.7. Results of labeling studies.

Methylerythritol Phosphate Pathway to IPP and DMAPP

NMR studies[14,17] have shown that most eukaryotes and archaebacteria operate using a "mevalonate-independent" or methylerythritol phosphate (MEP) pathway (**Scheme 1.2**). The MEP pathway begins with the condensation of glyceraldehyde phosphate and pyruvate to form 1-deoxy-D-xylulose-5-phosphate (DXP) catalyzed by DXP synthase. DXP is then rearranged and reduced to methylerythritol phosphate (MEP) by MEP synthase. This is the first committed step in the pathway. The pathway was named after this intermediate.



Scheme 1.2. Methylerythritol Phosphate (MEP) Pathway.

In the second committed step of the MEP pathway, MEP is converted to 4-diphosphocytidyl-2-*C*-methyl-D-erythritol (CDP-ME) by CDP-ME synthase. In the third step, CDP-ME is phosphorylated to 2-phospho-4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol (CDP-MEP) by CDP-ME kinase. CDP-MEP is then converted into 2-C-methyl-D-erythritol-2,4-cyclodiphosphate (cMEPP) by cMEPP synthase. A reductive ring opening of cMEPP provides 4-hydroxy-dimethylallyl-diphosphate (HDMAPP). IspH catalyzes the final step of the MEP pathway – formation of IPP and DMAPP.

There is only one potent inhibitor reported to date that specifically blocks the MEP pathway. Fosmidomycin inhibits the MEP synthase activity with an IC_{50} value of 8.2 nM and a K_i value of 38 nM.[18] The hydroxamic acid isostere of fosmidomycin and its methyl derivative are also potent inhibitors of MEP synthase.[19] Some modifications of fosmidomycin as FR900098 and 33289 have been reported and now their potency is being investigated.[20] Animals do not posses the enzymes found in the MEP pathway.[21] Thus, those enzymes are attractive targets for the development of herbicides and antimicrobial agents.

Crossover of MVA and MEP Pathways

Utilization of both pathways simultaneously was found only in higher plants and some microorganisms.[22] Their simultaneous functioning within the same cell raises questions of regulation and coordination of pathways. In the pathenogenic bacterium *Listeria monocytogenes*, both pathways are present, so the inhibition of any one of them does not interfere with organism survival.[23] In *Streptomices aeriouvifer* and *Actinoplanes*

sp. *A40644*, isoprenoids are synthesized by MEP in earlier growth stages, and by MVA at latter stages.[24] It is suggested that in *Streptomyces* the primary isoprenoid metabolites are from MEP, while the secondary ones are from MVA.[23]

A different situation is found in plants. In chloroplasts of plant cells, isoprenoids are synthesized from MEP, while in cytoplasm, they are from MVA. Therefore, although both pathways are present at the same time, they operate in separate compartments.[25]

Distribution of MEP and MVA Pathways

The nucleic acid sequences of about 150 microbial species afford a relatively detailed picture of the occurrence of MVA and MEP pathways.[26] All known archaeal genomes contain genes are similar to three MVA pathway genes: 3-hydroxy-3-methylglutaryl-CoA synthase (*hmgs*), 3-hydroxy-3-methylglutaryl-CoA reductase (*hmgr*), and mevalonate kinase (*mk*). On the other hand, genes that code for phosphomevalonate kinase (Pmk), diphosphomevalonate decarboxylase (Dpmd), and isopentenyl diphosphate isomerase (IDI type I) from eukaryotes are not found. *Borrelia burgdorferi* and *Staphylococcus aureus* carry complete sets of MVA pathway genes, but do not have the gene for IDI type I. Instead, they carry a newly discovered gene for a type II isopentenyl diphosphate isomerase (IDI type II). The genome of *Listeria monozytogenes* contains complete sets of MVA and MEP pathway genes along with the gene for IDI type II. *Streptomyces coelicolor* has a complete set of MEP pathway genes. On the other hand, some *Streptomyces sp.* possess enzymes of the mevalonate pathway. No genes with similarity to those of either the MVA pathway or the MEP pathway were

found in *Mycoplasma genitalium* or *Rickettsia provazekii.* Some eubacteria with full sets of MEP pathway genes, e.g. *E. coli*, *Synechocystis* sp., *B. subtilis, Deinococcus radiodurans*, and *M. tuberculosis*, possess gene orthologs for either IDI type I or II. Since IPP and DMAPP can be synthesized independently by the catalytic action of the IspH protein, it can be assumed that the isomerases serve as nonessential pathway proteins in organisms. The deletion of *idiI* mutants in *E. coli* was found to be not lethal.[26]

DXP Synthase (DXS)

The initial step in the MEP pathway is the formation of 1-deoxy-D-xylulose 5-phosphate by the condensation of pyruvate and D-glyceraldehyde 3-phosphate, catalyzed by DXP synthase (**Scheme 1.2**). The *dxs* gene was first cloned from *E. coli*. This enzyme has a typical thiamine-binding motif and needs both thiamine and a divalent cation such as Mg^{2+} or Mn^{2+} for activity.[27] DXP is a biosynthetic intermediate not only for IPP and DMAPP but also for thiamine and pyridoxol. This fact means that DXP synthase is a key enzyme not only for the MEP pathway but also for thiamine and pyridoxol biosynthesis. Only recently, structures of the *Escherichia coli* and *Deinococcus radiodurans* enzymes were reported.[28] The DXS subunit consists of three domains, each similar to equivalent domains of transketolase, which catalyzes a similar reaction. The X-ray structure of DXS is shown in **Figure 1.8**. However, the arrangement of domains is different in the two enzymes. Like transketolase, the DXS coenzyme complex is expected to form a covalent adduct between thiamine diphosphate and the C2 of pyruvate.[29] Unlike transketolase where the active site is located at the dimer interface, the active site of DXS is created

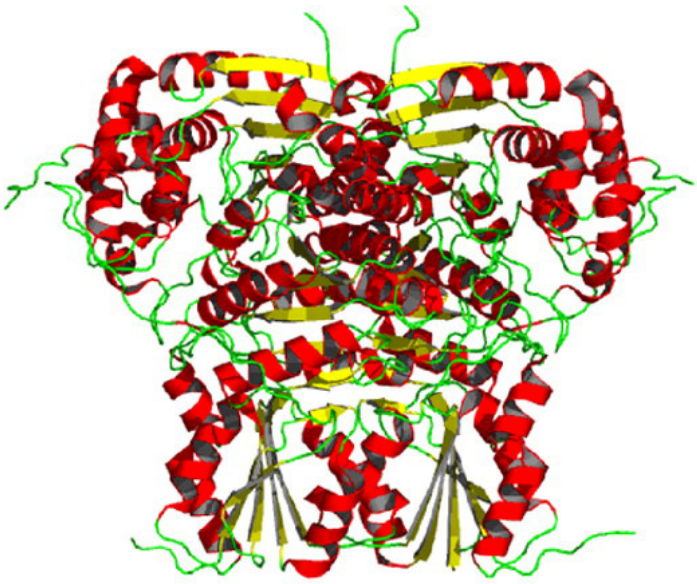


Figure 1.8. X-ray structure of DXS.

with the cofactor buried within a monomer, placing the thiazolium moiety at the base of a polar cavity. The structures required to confirm aspects of specificity for and binding of substrate are lacking.

MEP Synthase (IspC)

Rohmer and coworkers found that 2-*C*-methylerythritol was incorporated into the side chain of ubiquinone in *E. coli*.[30] The studies also showed that MEP is synthesized in a single step by rearrangement of DXP followed by reduction of the aldehyde by NADPH (**Scheme 1.2**).[31] This is the first committed step in the pathway. The crystal structure of MEP synthase from *E. coli* complexed with NADPH was reported as a homodimer (**Figure 1.9**).[32] The monomer structure is composed of three domains: an N-terminal NADPH binding domain, a central domain, and a C-terminal α-helical domain. The

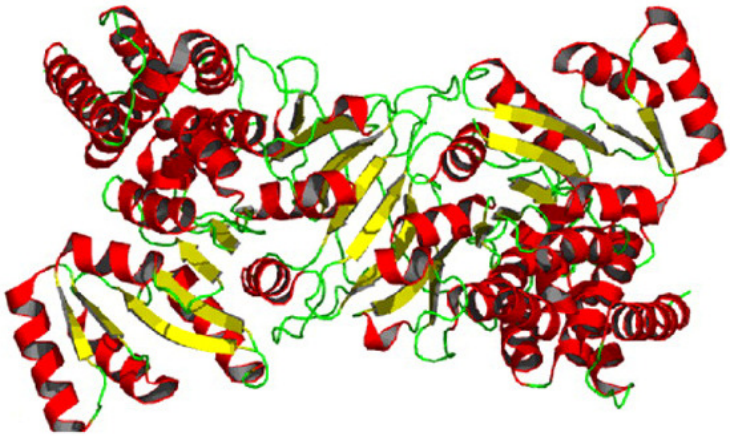


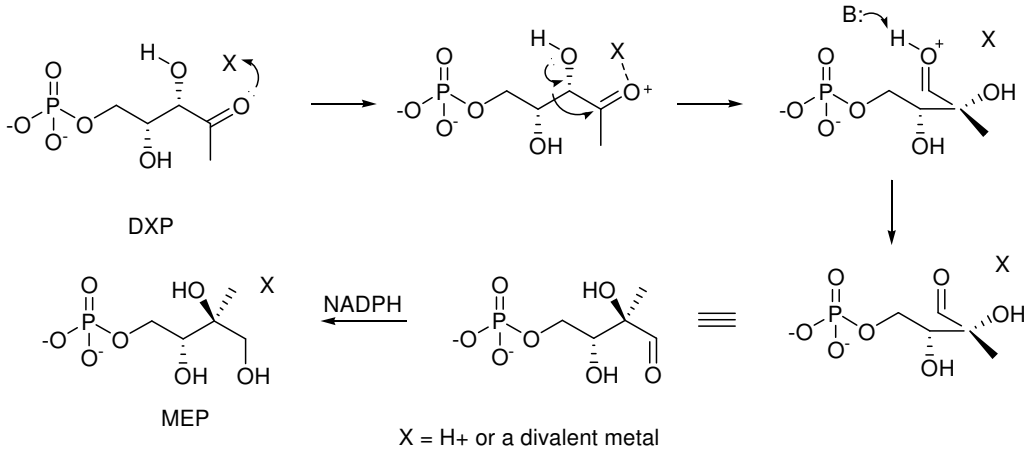
Figure 1.9. X-ray structure of IspC.

crystal structure indicated that the important amino acid residues for catalysis and DXP binding are located in a catalytic pocket in the central domain with N- and C-terminal domains protruding into the pocket. The crystal structure also suggested that a loop portion between His-209 through Met-214 can be a "hatch" that closes the active site when the substrate entered the catalytic pocket. A hydrogen bond between His-209 and the phosphate group in DXP could close the hatch to fix the substrate so that the reaction proceeds effectively.[33,34] Koppish *et al.* reported that a divalent metal was required for activity.[35]

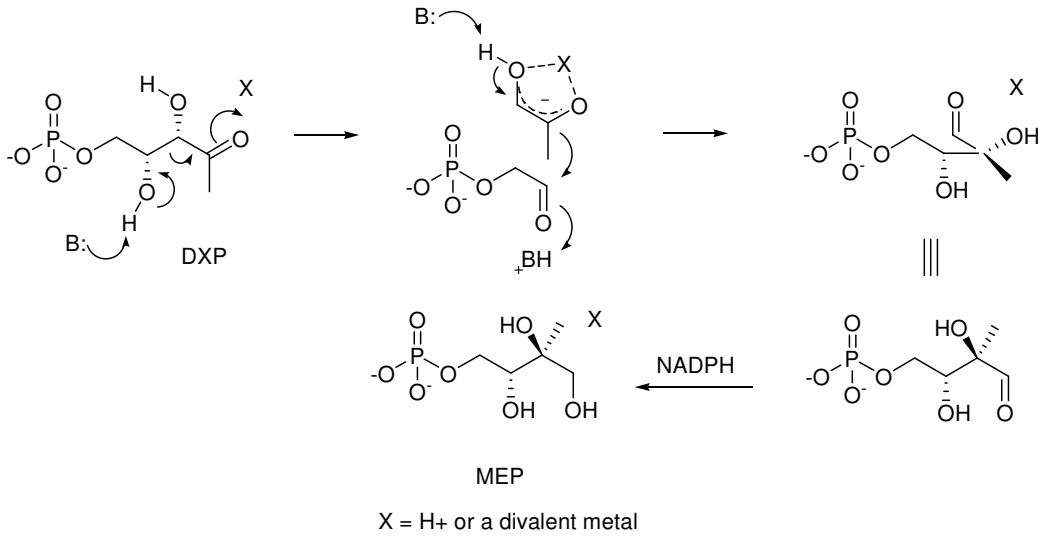
The crystal structure with fosmidomycin (inhibitor of MEP synthase) reveals hydrogen bonding interactions between the phosphonate group and Ser-186, Ser-222, Asn-227, Lys-228. MEP was independently synthesized by Koppish *et al.* from 1,2-propanediol.[36]

There are two mechanisms proposed for the conversion of DXP to MEP, α-ketol (**Scheme 1.3**) and retroaldol rearrangements (**Scheme 1.4**).[37] The α-ketol mechanism involves the deprotonation of the hydroxyl group at C3 of DXP, followed by the migration of the phosphate-bearing C2 subunit to afford an aldehyde, which is then reduced to form MEP. Examples of reactions proceeding by a retroaldolization/aldolization type mechanism instead of α-ketol rearrangement are also found in the literature. An example is the reaction catalyzed by the ribulose 5-phosphate 4-epimerase.[38]

In the retroaldol mechanism (**Scheme 1.4**), a base deprotonates a hydroxyl group at C4, followed by cleavage of the C3-C4 bond to give two species - glycoaldehyde phosphate and hydroxyacetone enolate. Subsequent aldolization gives identical aldehyde intermediate generated by an α-ketol rearrangement, followed by NADPH reduction to give MEP. Fox *et al.* prepared a set of fluoro DXP analogues as substrates/inhibitors for MEP synthase.[37] In those analogues, the three methyl hydrogens at C1 of DXP were



Scheme 1.3. Possible mechanism for MEP synthase - α-ketol rearrangement.



Scheme 1.4. Possible mechanism for MEP synthase - retroaldol rearrangement.

sequentially replaced by a fluorine atom to give CF-DXP, CF_2-DXP, and CF_3-DXP (**Figure 1.10**). CF_2-DXP and CF_3-DXP were modest inhibitors of MEP synthase with IC_{50} values of 2.0 and 3.4 mM, respectively. CF-DXP was a good substrate (k_{cat} = 38 s^{-1}, K_m = 227 μM) with a turnover rate similar to that of the natural substrate. These results support a retroaldol mechanism rather than an α-ketol rearrangement for the enzyme-catalyzed conversion of DXP to MEP.

Additionally, Walker *et al.* synthesized and evaluated a series of DXP analogues as inhibitors of *E. coli* MEP synthase.[39] The methyl group in DXP was replaced by hydroxyl, hydroxylamino, methoxy, and amino moieties, respectively.

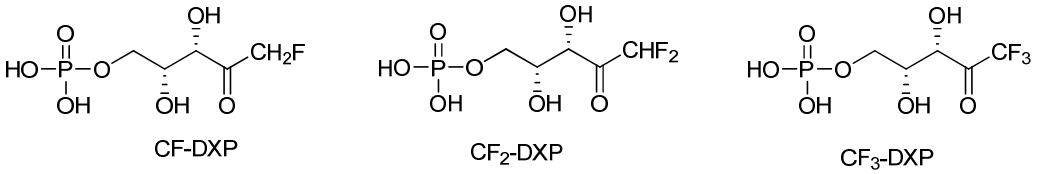


Figure 1.10. Fluoro analogues of DXP.

Also, the acetyl moiety in DXP was replaced by hydroxymethyl and aminomethyl groups (**Figure 1.11**). These compounds were designed to coordinate to the active site divalent metal in MEP synthase. The carboxylate, methyl ester, amide, and alcohol analogues were inhibitors with IC_{50}'s ranging from 0.25 to 1.0 mM. The hydroxamic acid and amino analogues did not inhibit the enzyme. These results show that the MEP synthase has strict requirements for inhibitors or substrates. The most potent members of the group closely approximated the size of DXP and contained functional groups that could bind to the divalent metal in the active site. Changes that compromise either of these two features result in a substantial decrease of potency.

CDP-ME Synthase (IspD)

The *IspD* gene product converts MEP into 4-(cytidine 5'-diphospho)-2-*C*-methyl-D-erythritol (CDP-ME) using CTP (**Scheme 1.2**). This enzyme was designated CDP-ME

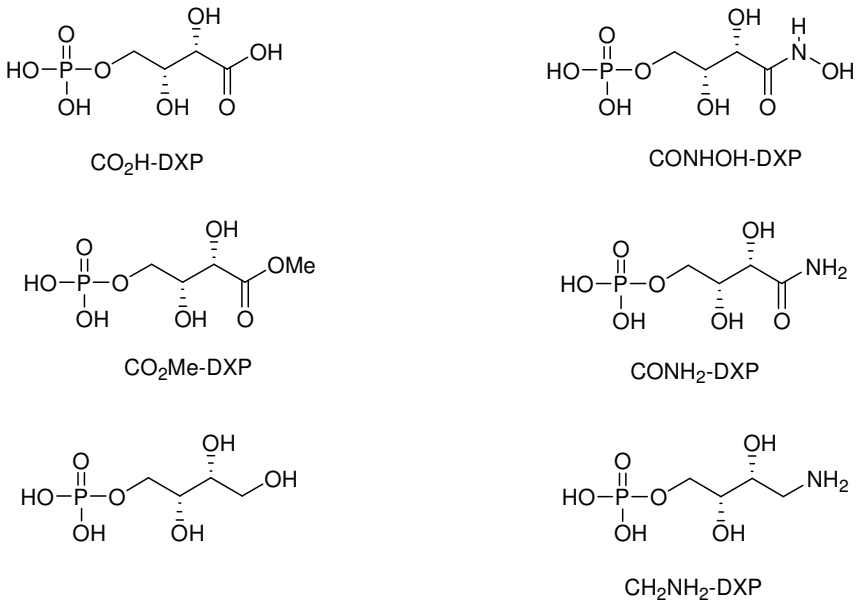


Figure 1.11. Analogues of DXP synthase.[40]

Noel and coworkers have reported three-dimensional dimer structures of CDP-ME synthase in the apo form and the form bound to CTP-Mg^{2+} or CDP-ME-Mg^{2+}.[41] The X-ray structure is shown in **Figure 1.12**.

The subunit is a single domain α/β structure constructed around a seven-stranded twisted β-sheet into which is inserted an extended "β-arm." Two arms form a dimer interface that involves numerous hydrogen bonds and salt bridges. Interactions of the protein with the cytosine moiety of CTP include coordination of nitrogen with Ser-88, hydrogen bonds between the carbonyl group and Ala-14, and Ala-15 backbone amides. Arg-20 interacts with the α- and γ-phosphates, Lys-27 plays an essential role in catalysis, and Lys-213 could act as an electrostatic guide for the phosphate function of MEP prior to nucleophilic attack on CTP. The product of catalysis is stabilized by interaction with a side chain of Thr-165. Several bacteria were found to have a bifunctional fusion genes consisting of *IspD* and *IspF* domains. In *Agrobacterium tumefaciens*, the *ispD* and *ispF* genes are fused to encode a bifunctional enzyme that catalyzes the first (synthesis of 4-

Figure 1.12. X-ray structure of IspD.

diphosphocytidyl-2-*C*-methyl-D-erythritol) and third (synthesis of 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate) steps. Lherbet *et al.*[42] showed with sedimentation velocity experiments that the bifunctional IspDF and IspE proteins associate in solution. However, a time course experiment indicated that the intermediates are not transferred from the IspD active site in IspDF to the active site of IspE or from the active site in IspE to the active site of the IspF module of IspDF. The CDP-ME was also synthesized independently by Koppisch *et al.*[43] by coupling of free phosphoacid of MEP with cytidine 5'-monophosphate using a protocol originally developed for synthesis of base-sensitive nucleoside diphosphate sugars.[44]

CDP-ME Kinase (IspE)

The *IspE* gene product converts CDP-ME into 2-phospho-4-(cytidine 5'-diphospho)-2-*C*-methyl-D-erythritol (CDP-MEP) in the presence of ATP (**Scheme 1.2**).[45] The dimer structure of this enzyme in its apo form recently was reported without bound substrates.[46] It was proposed that Asn-58 and Lys-83 are involved in binding ATP and that Ser-95 and Asp-125 coordinate to Mg^{2+}. It was suggested that the Asp-125 oxygen, activated by Lys-8, eliminates the proton from the hydroxyl group at C2 position of CDP-ME. The X-ray structure is shown on **Figure 1.13**.

The enzyme displays the characteristics of a GHMP kinase α/β fold, with cofactor and substrate-binding domains. The Lys-10 and Asp-141 pair forms hydrogen bonds with substrate and polarizes the hydroxyl group to facilitate proton removal by Asp-141.

Figure 1.13. X-ray structure of IspE.

The γ-phosphate of the cofactor is transferred to CDP-ME to produce 4-diphosphocytidyl-2*C*-methyl-D-erythritol-2-phosphate (CDP-MEP) and ADP. There is no evidence for divalent metal ion binding to the cofactor, and the cofactor purine forms hydrogen-bonding interactions that stabilize the less common *syn* orientation of the base with respect to the glycosidic bond.[47]

cMEPP Synthase (IspF)

The *IspF* gene product converts CDP-MEP into 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate (cMEPP) (**Scheme 1.2**). The formation of cMEPP was concomitant with elimination of CMP from CDP-MEP. The X-ray structure is shown in **Figure 1.14**. The crystal structure of cMEPP synthase from *E. coli* was reported independently by three groups.[48,49,50] The crystal structure presents a compact homotrimer in the asymmetric unit. The IspF subunit is a small single α/β domain and consists of a four-stranded mixed β-sheet on one side with three α helices on the other. Two metal coordination sites were reported in the structure.

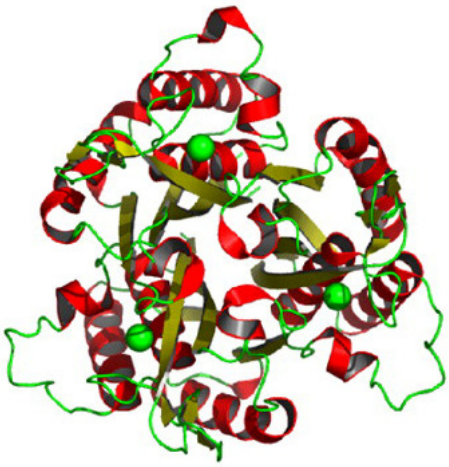


Figure 1.14. X-ray structure of IspF.

A Zn^{2+} site positions the phosphate group and facilitates the nucleophilic attack. A second metal Mn^{2+} coordinates the diphosphate group. In the site, Lys-130 forms a salt bridge with Asp-95, Ala-131 binds the cytidyl group, and Thr-133 forms hydrogen bonds with the phosphate of CDP or CMP upon release. The enzyme requires both cations for catalysis, one of which (Zn^{2+}) is always present in the active site.

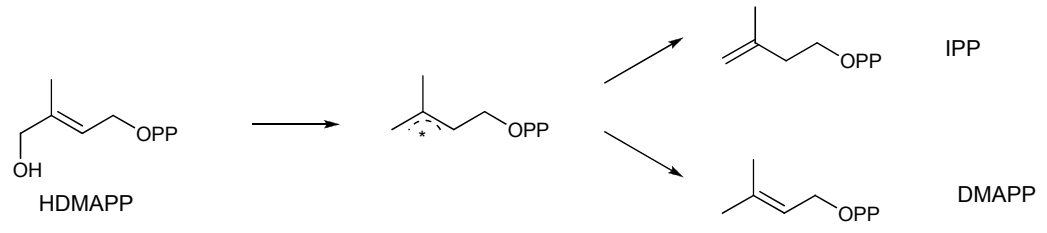
IspG and IspH Proteins

The two least studied enzymes in the MEP pathway, IspG and IspH, are described more closely in Chapter 2. cMEPP undergoes reduction and elimination to 4-hydroxy-3-methyl-2-(*E*)-butenyl-4-diphosphate (HDMAPP), followed by reduction to IPP and DMAPP (**Scheme 1.2**). An amino acid sequence comparison of the *E. coli* IspG protein with the orthologs from *M. tuberculosis* and *P. falciparum* shows a high degree of homology among the sequences.[51] These genes specify a cluster of three absolutely conserved cysteines[52] typical for iron–sulfur proteins such as the S-adenosyl methionine

dependent radical enzyme family. Optical spectra showed a shallow absorption maximum at about 410 nm which has been interpreted as evidence for an iron–sulfur cluster. The conserved cysteine residues are assumed to be involved in the binding of that cluster. The extraordinary sensitivity of the enzyme against molecular oxygen is well in line with the hypothesis that the iron–sulfur cluster is essential for the reaction mechanism. Since IspH protein as well as IspG protein contains three conserved cysteine residues, they are believed to be involved in catalysis or binding. The replacement of any of the cysteine residues reduced the catalytic activity of the IspH protein by a factor of more than 70,000. In order to eliminate the OH group, the formation of reactive intermediates, in which the symbol * is a reference to the unknown number of electrons (**Scheme 1.5**). The reaction could proceed *via* an anionic, radical, or a cationic allyl system. Both IPP and DMAPP can be obtained by the transfer of H^+, $H^\bullet$ or H^- to either the C2 or C4 position of the hypothetical intermediate.

Overview of Dissertation

Chapter 2 will present the synthesis of HDMAPP analogues, where the hydroxy group is substituted by amino and thio groups. Chapter 3 will present the synthesis of analogues for the MEP, where the primary hydroxy group is displaced with the amino group, the phosphate is substituted with thiophosphate, and the tertiary hydroxy group is



Scheme 1.5. A generalized structure for the hypothetical intermediate(s) at the branching point of the IspH-mediated reaction.

displaced by thiol. The LCMS and radioactive isotope labeling experiments are conducted to check the analogue activity.

References

1. Thulasiram, H. V.; Erickson, H. K.; Poulter, C. D. *Science*, **2007**, *316*, 73-75.

2. Cane, D. E. *Comprehensive Natural Products Chemistry*; Barton D., Nakanishi, K., Meth-Cohn, O., Ed. Elsevier, Oxford, UK, 1999, vol. 2, 1-11.

3. Dolence, J. M. Poulter, C. D. *Comprehensive Natural Products Chemistry*; O. Meth-Cohn, Ed. Elsevier, Oxford, UK, 1999, vol. 5, 18473–18500.

4. .Rohmer, M. *Comprehensive Natural Products Chemistry*; Barton D., Nakanishi, K., Meth-Cohn, O., Ed. Elsevier, Oxford, UK, 1999, vol. 2, 45-67.

5. Rohmer, M.; Sutter, B.; Sahm, H. *J. Chem. Commun*., **1989**, 1471-1472.

6. Eisenreich, W.; Menhard, B.; Hylands, P.; Zenk, M.; Bacher, A. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 6431-6436.

7. Schwender, J.; Seeman, M.; Lichtenthaler, H.; Rohmer, M. *Biochem. J.*, **1996**, *316*, 73-80.

8. Banthorpe, D. V.; Charlwood, B. V.; Francis, M. J. O. *Chem. Rev*., **1972**, *72*, 115–155.

9. Bach, T. J.; Motel, A.; Weber, T. *Rec. Adv. Phytochem*., **1990**, *24*, 1–82.

10. Rohmer, M. *Pure Appl. Chem.*, **2003**, *75*, 375-387.

11. Bach, T.; Motel, A.; Weber, T. *Rec. Adv. Phytochem*., **1990**, *24*, 1-82.

12. Rohmer, M.; Bouvier, P.; Ourisson, G. *Proc. Natl. Acad. Sci. USA*, **1979**, *76*, 847-851.

13. Rohmer, M.; Bouvier-Nave, P.; Ourisson, G. *J. Gen. Microbiol*., **1984**, *130*, 1137-1150.

14. Flesch, G.; Rohmer, M. *Eur. J. Biochem*., **1988**, *175*, 405-411.

15. Conway T. *FEMS Microbiol Rev*., **1992**, *1*, 1–27.

16. Rohmer, M.; Knani, M.; Simonin, P.; Sutter, B.; Sahm, H. *Biochem. J.,* **1993**, *295*, 517-524.

17. Eisenreich, W.; Schwarz, M.; Cartayrade, A.; Arigoni, D.; Zenk, M.; Bacher, A. *Chem. Biol*., **1998**, *5*, 221-233.

18. Kuzuyama, T.; Shimizu, T.; Takahashi, S.; Seto, H. *Tetrahedron Lett.*, **1998**, *39*, 7913-7916.

19. Kuntz, L.; Tritsch, D.; Grosdemange-Billiard, C.; Hemmerlin, A.; Willem, A.; Bach, T. J.; Rohmer, M. *Biochem. J.*, **2005**, *386*, 127-135.

20. Jomaa, H.; Wiesner, J.; Sanderbrand, S.; Altincicek, B.; Weidemeyer, C.; Hintz, M.; Türbachova, I.; Eberl, M.; Zeidler, J.; Lichtenthaler, H. K.; Soldati, D.; Beck, E. *Science,* **1999**, *285*, 1573 – 1576.

21. Lange, B.; Rujan, T.; Martin, W.; Croteau, R. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 13172-13177.

22. Kuzuyama, T.; Takagi, M.; Takahashi, S.; Seto, H. *J. Bacteriol.*, **2000**, *182*, 891-897.

23. Hamano, Y.; Dairi, T.; Yamamoto, M.; Kuzuyama, T.; Itoh, N.; Seto, H. *Biosci. Biotechnol. Biochem.,* **2002**, *66*, 808-816.

24. Seto, H.; Orihara, N.; Furihata, K. *Tetrahedron Lett.*, **1998**, *39*, 9427-9500.

25. Schwender, J.; Zeidler, J.; Groner, R.; Muller, C.; Focke, M.; Braun, S.; Lichtenthaler, F.; Lichtenthaler, H. *FEBS Lett.*, **1997**, *414*, 129-134.

26. Eisenreich, W.; Bacher, A.; Arigoni, D.; Rohdich, F. *Cell. Mol. Life Sci.*, **2004,** *61*, 1401–1426.

27. Kuzuyama, T.; Seto H. *Nat. Prod. Rep.*, **2003**, *20*, 171–183.

28. Xiang, S.; Usunow, G.; Lange, G.; Busch, M.; Tong, L. *J. Biol. Chem.*, **2007**, *282*, 2676–2682.

29. Fiedler, E.; Thorell, S.; Sandalova, T.; Golbik, R.; Konig, S.; Schneider, G. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 591–595.

30. Duvold, T.; Cali, P.; Bravo, J.; Rohmer, M. *Tetrahedron Lett.*, **1997**, *38*, 6181-6184.

31. Takahashi, S.; Kuzuyama, T.; Watanabe, H.; Seto, H. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*, 9879-9884.

32. Estevez, J. M.; Cantero, A.; Reindl, A.; Reichler, S.; Leon, P. *J. Biol. Chem*., **2001**, *276*, 22901–22909.

33. Yajima, S.; Nonaka, T.; Kuzuyama, T.; Seto H.; Ohsawa, K. *J. Biochem.*, **2002**, *131*, 313-317.

34. Reuter, K.; Sanderbrand, S.; Jomaa, H.; Wiesner, J.; Steinbrecher, I.; Beck, E.; Hintz, M.; Klebe G.; Stubbs, M. T. *J. Biol. Chem.*, **2002**, *277*, 5378-5384.

35. Koppisch, A.; Fox, D.; Blagg, B.; Poulter, C. *Biochemistry*, **2002**, *41*, 236-243.

36. Koppisch, A. T.; Blagg, B. S. J.; Poulter, C. D. *Org. Lett.*, **2000**, *2*, 315-317.

37. Fox, D. T.; Poulter, C. D. *J. Org. Chem.*, **2005**, *70*, 1978–1985.

38. Lee, L. V.; Vu, M. V.; Cleland, W. W. *Biochemistry*, **2000**, *39*, 4808–4820.

39. Walker, J. R.; Poulter, C. D. *J. Org. Chem.,* **2005**, *70*, 9955 -9959.

40. Kuzuyama, T.; Takagi, M.; Kaneda, K.; Dairi T.; Seto, H. *Tetrahedron Lett.*, **2000**, *41*, 703-706.

41. Richard, S. B.; Bowman, M. E.; Kwiatkowski, W.; Kang, I.; Chow, C.; Lillo, A. M.; Cane, D. E.; Noel, J. P. *Nature Struc. Biol.*, **2001**, *8*, 641-648.

42. Lherbet, C.; Pojer, F.; Richard, S. B.; Noel, J. P.; Poulter, C. D. *Biochemisry*, **2006**, 45, 3548-3553.

43. Koppisch, A. T.; Poulter, C. D. *J. Org. Chem.*, **2002**, *67*, 5416-5418.

44. Marlow, A. L.; Kiessling, L. L. *Org. Lett.*, **2001**, *3*, 2517-2519.

45. Kuzuyama, T.; Takagi, M.; Kaneda, K.; Dairi T.; Seto, H. *Tetrahedron Lett.*, **2002**, *41*, 2925-2928.

46. Wada, T.; Kuzuyama, T.; Satoh, S.; Kuramitsu, S.; Yokoyama, S.; Unzai, S.; Tame, J. R,; Park, S. Y.; *J. Biol. Chem.*, **2003**, *278*, 30022–30027.

47. Hunter, W. N. *J. Biol. Chem*., **2007**, *282*, 21573–21577.

48. Richard, S.; Ferrer, J.-L.; Bowman, M.; Lillo, A.; Tetzlaff, C.; Cane, D.; Noel, J. *J. Biol. Chem.*, **2002**, *277*, 8667-8672.

49. Kemp, L.; Bond, C.; Hunter, W. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 6591-6596.

50. Steinbacher, S.; Kaiser, J.; Wungsintaweekul, J.; Hecht, S.; Eisenreich, W.; Gerhardt, S.; Bacher A.; Rohdich, F. *J. Mol. Biol.*, **2002**, *316*, 79-88.

51. Laupitz, R.; Hecht, S.; Amslinger, S.; Zepeck, F.; Kaiser, J.; Richter, G.; Schramek, N.; Steinbacher, S.; Huber, R.; Arigoni, D.; Bacher, A.; Eisenreich, W.; Rohdich, F. *Eur. J. Biochem.*, **2004**, *271*, 2658–2669.

52. Hecht, S.; Eisenreich, W.; Adam, P.; Amslinger, S.; Kis, K.; Bacher, A.; Arigoni, D.; Rohdich, F. *Proc. Natl. Acad. Sci. USA*, **2001**, *98*, 14837–14842.

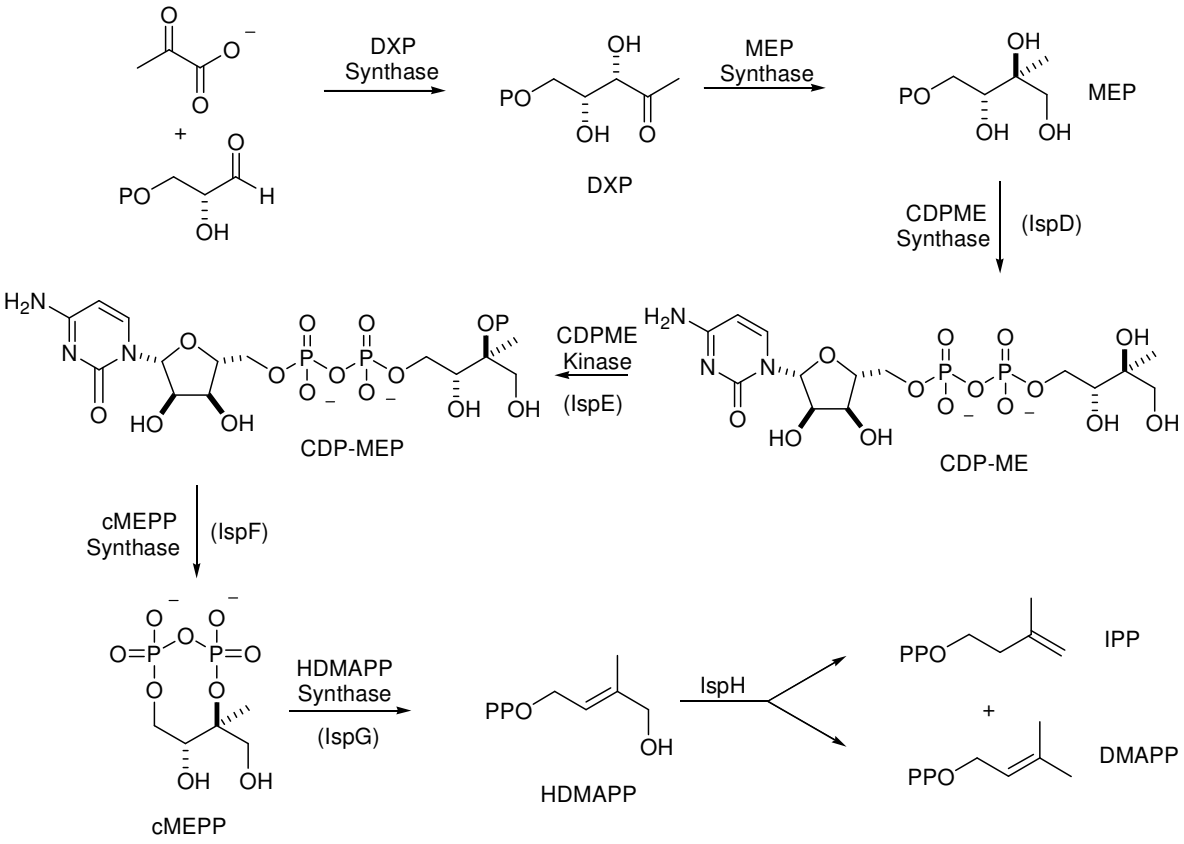
CHAPTER 2

SYNTHESIS AND EVALUATION OF 4-HYDROXY-DIMETHYLALLYL DIPHOSPHATE ANALOGUES

Background

The MEP pathway (**Scheme 2.1**) begins with the condensation of glyceraldehyde phosphate and pyruvate to form 1-deoxy-D-xylulose-5-phosphate (DXP) catalyzed by DXP synthase. DXP is then rearranged and reduced by MEP synthase to produce methylerythritol phosphate (MEP). MEP is converted into methylerythritol cyclodiphosphate (cMEPP) by the consecutive actions of three enzymes: CDPME synthase, CDPME kinase, cMEPP synthase. A reductive ring opening of cMEPP provides hydroxydimethylallyl diphosphate (HDMAPP). The IspH catalyzes the final step of the MEP pathway – formation of IPP and DMAPP. It is interesting to note that in contrast to the mevalonate (MVA) pathway, organisms utilizing the MEP pathway do not require IPP isomerase activity, although the enzyme is found in most of these organisms (**Scheme 2.1**).[1]

This chapter is dedicated to studies of the last steps of the MEP pathway, reactions catalyzed by IspG and IspH. Our goal was to prepare analogues for 4-hydroxydimethylallyl diphosphate (HDMAPP) as inhibitors or substrates for IspH.



Scheme 2.1. Methylerythritol Phosphate (MEP) Pathway.

Additionally, we are interested to see if these analogues will be product inhibitors for IspG, since both proteins are similar to some extent. Overall, the reactions catalyzed by IspG and IspH involve elimination and reduction. The nucleotide sequences of the genes that encode the proteins have conserved cysteine residues in a pattern consistent with formation of a Fe-S cluster.[2] This observation is important, since the enzymes are oxygen sensitive and require inert atmosphere for their operation. Both proteins have three conserved cysteines which form an Fe-S cluster characterized by UV-vis absorption with a maximum at 410 nm.[3] In the case of the IspG, this cluster was detected by Mössbauer spectroscopy, and in case of the IspH enzyme the same cluster was identified

by EPR spectroscopy.[4] The Fe-S cluster could play a role as a Lewis acid or as a reducing agent involved in $1e^-$ transfer.

The flavodoxin encoding gene *fldA* is essential for the survival of *E. coli*.[3] It was proposed to be involved, along with NADPH, in the reduction of the oxidized $[4Fe\text{-}4S]^{2+}$ cluster to the reduced $[4Fe\text{-}4S]^{1+}$ state in IspG and IspH.[5] Rohdich *et al.* discovered that 40 μM flavodoxin and 12 μM flavodoxin reductase were required for maximum activity of IspH expressed from *E. coli*. Among the total of four cysteines, IspH has three absolutely conserved cysteine residues at positions 12, 96, and 197. Replacement of any of the conserved cysteines reduced the catalytic activity by a factor of more than 70,000.[6]

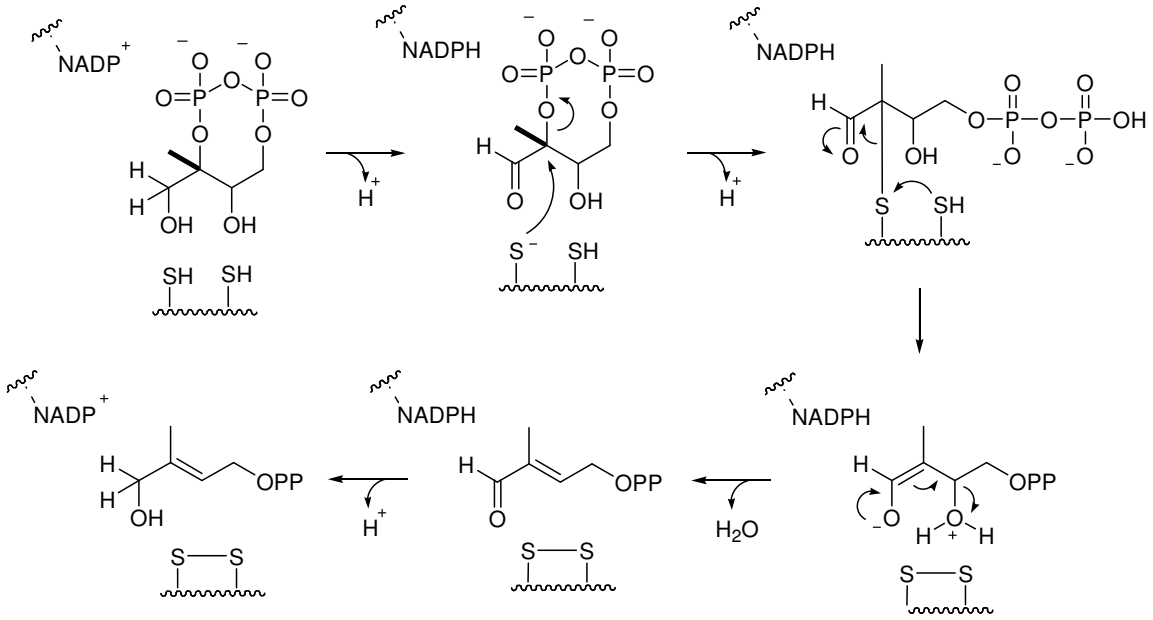
IspG Protein

Rohmer and coworkers[7] reported that cMEPP was a substrate for the IspG enzyme. They engineered *E. coli* cells with deleted *dxr* and overexpressed *IspG* genes. After incubation of these cells with tritium labeled ME, accumulation of radiolabeled HDMAPP was observed, which suggested that cMEPP was a substrate of IspG. Incubation of ^{14}C-labeled cMEPP with a crude lysate from *E. coli* cells with exhanced loads of the IspG enzyme led to formation of only one radioactive molecule - 4-hydroxydimethyallyl alcohol, which was a hydrolysis product of HDMAPP by phosphatases. An incubation of cMEPP in the presence of phosphatase inhibitors gave HDMAPP, which was compared with chemically synthesized HDMAPP and was accepted as the reaction product of the IspG catalyzed reaction.[8] HDMAPP was also synthesized independently by Fox *et al*.[9] It was also shown by Kollas *et al.*[10] that the IspG converts cMEPP to HDMAPP without the need for any additional protein factors by

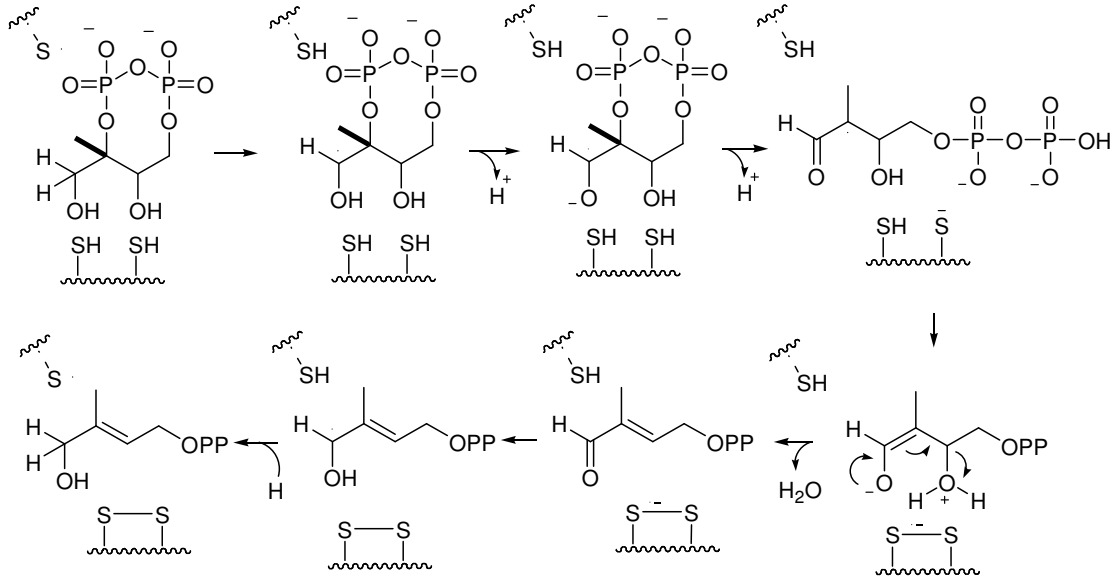
using dithionite as an artificial electron donor. As it was mentioned earlier, the reaction catalyzed by the IspG formally involve a two-electron reduction that requires cleavage of two carbon-oxygen bonds. It was shown in deuterium labeling studies with *E. coli* that all the C-H bonds of the cMEPP are conserved in IPP and DMAPP.[11,12] Two mechanisms were proposed to account for these observations (**Scheme 2.2** and **Scheme 2.3**).[13]

A $2e^-$ transfer mechanism is presented in **Scheme 2.2**. In the first step, $NADP^+$ oxidizes the primary alcohol to an aldehyde. In the second step, a thiolate displaces the diphosphate at C3, leading to a covalent complex between the Fe-S cluster and the substrate. In the next step, a disulfide bond is formed with concomitant cleavage of the thioether to give an enolate. The enolate is converted to its aldehyde form upon protonation of the β hydroxy group followed by displacement of a water molecule. The alcohol moiety is restored by reduction with NADPH in the last step to produce HDMAPP. An alternative $1e^-$ transfer mechanism is shown in **Scheme 2.3**. The thiyl radical derived from the third conserved cysteine residue removes a hydrogen atom from C4 of cMEPP to give an intermediate that is predisposed for diphosphate elimination.

In the following step, formation of the aldehyde is accompanied by elimination of the diphosphate leading to the formation of a tertiary radical. Transfer of one electron from a thiol generates a disulfide, and leads to an enolate, which in turn loses water to form an α,β-unsaturated aldehyde. Eventual reduction of the aldehyde gives the product HDMAPP.



Scheme 2.2. Hypothetical 2e$^-$ mechanism for IspG.



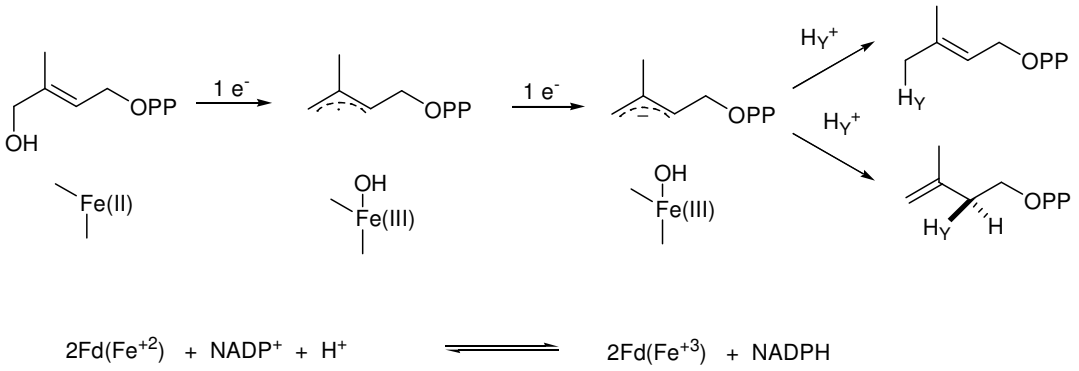
Scheme 2.3. An alternative hypothetical 1 e$^-$ mechanism for the IspG.

IspH Protein

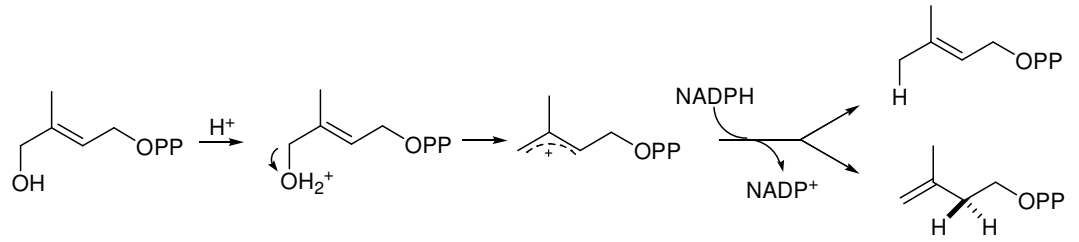
A similar approach to the described for the IspG studies was used to investigate the *IspH* gene product. Jomaa and coworkers engineered *E. coli* strain which contained the three genes required to convert mevalonate to IPP and deleted *IspH* gene.[14] This deletion was lethal. However, the strain could be rescued by addition of mevalonate to the medium. Under these conditions, formation of 4-hydroxydimethylallyl diphosphate was detected by spectroscopic methods. The role of IspG and IspH was determined by incorporation of [U-$^{13}C_6$]DX in *E. coli* strain with overexpressed enzymes of the MEP pathway, followed by analysis of the accumulated metabolites by ^{13}C NMR of the crude cell free extracts. Overexpression of *IspDEF* genes resulted in the accumulation of cMEPP product. Additional expression of IspG led to the formation of HDMAPP, while expression of IspG and IspH gave IPP and DMAPP in a 5:1 ratio.[15] Thus, cMEPP and HDMAPP are substrates for IspG and IspH, respectively.

Recombinant IspH from the thermophilic eubacterium *Aquifex aeolicus* was purified by Jomaa and coworkers.[16] The researchers developed a spectrophotometric assay to determine the steady-state kinetic parameters of IspH. A maximal specific activity of 6.6 ± 0.3 μmol $min^{-1}mg^{-1}$ protein was determined at pH 7.5 and 60 °C. k_{cat} for the IspH was 3.7 ± 0.2 s^{-1} and the *K*m value for HDMAPP was 590 ± 60 μM.

IspH and IspG proteins contain three conserved cysteine residues, typical of protein with an Fe-S cluster, believed to be involved in catalysis or binding.[15] The final step of biosynthesis of IPP and DMAPP in the MEP pathway requires replacement of a hydroxy group by a hydrogen atom. There are two possible mechanisms for this transformation (**Scheme 2.4, and Scheme 2.5**). A mechanism for this reaction proposed



Scheme 2.4. Hypothetical 1e$^-$ mechanism for the IspH reaction.



Scheme 2.5. Hypothetical protonation mechanism for the IspH reaction.

by Rohdich *et al.* involves elimination of the hydroxyl group to generate an allylic cation, which is then reduced to an allylic anion and protonated either at carbon C2 or C4 to give IPP or DMAPP, respectively (**Scheme 2.4**).[17] Although it was shown that the transferred hydrogen is most likely a proton by overall six-fold deuterium discrimination in favor of ^{1}H in 90% heavy water with IspH from *E. coli*,[18] the reaction might proceed through protonation of C4 hydroxyl group with subsequent generation of a tertiary carbocation, followed by hydride transfer from NADPH (**Scheme 2.5**).

Rationale

We proposed two substrate analogues to test these proposed mechanisms. This chapter describes the synthesis and enzymatic activity evaluation of substrate analogues:

aminodimethylallyl diphosphate (NDMAPP) and thiodimethylallyl diphosphate (SDMAPP) for hydroxydimethylallyl diphosphate (HDMAPP) (**Figure 2.1**).

Our intent was to demonstrate how changes in the natural substrate affect the enzymatic conversion of the alternative substrates to the final products in the MEP pathway, IPP and DMAPP by IspH. We were also interested in the activity of the above-mentioned molecules with IspG to see if they could serve as product inhibitors. NDMAPP can be active as a free amine in the mechanism, where Fe^{2+} from the iron-sulfur cluster acts as a Lewis acid and a radical is formed (**Scheme 2.6**). Additional electron transfer generates an anion which is protonated to give either IPP or DMAPP. From another mechanism, the NDMAPP amino group can be protonated by acid, followed by ammonia cleavage (**Scheme 2.6**). NH_3^+ surrogates for OH_2^+, but it is a much poorer leaving group which may result in no or slow cleavage. SDMAPP can be active by slowing down the elimination step in the mechanism, where Fe^{2+} from the Fe-S cluster acts as a Lewis acid and forms an allylic radical. Additional electron transfer generates an

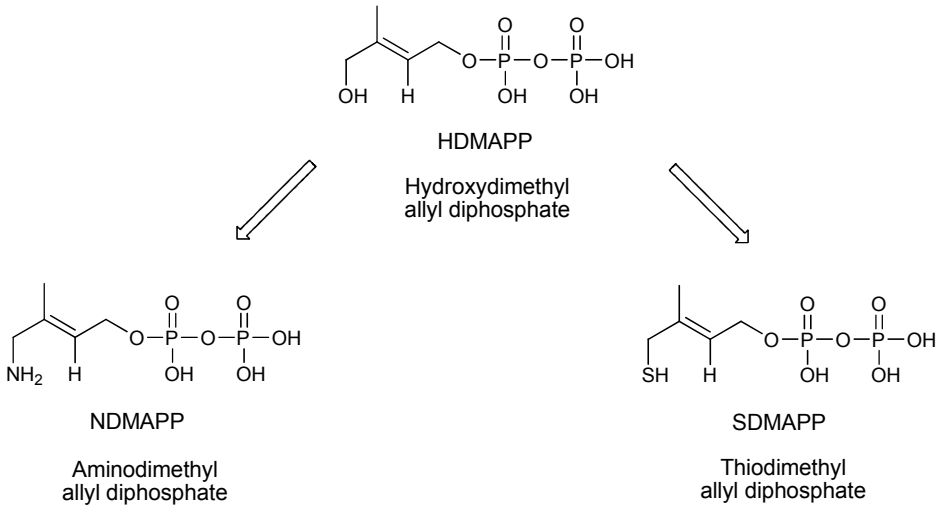
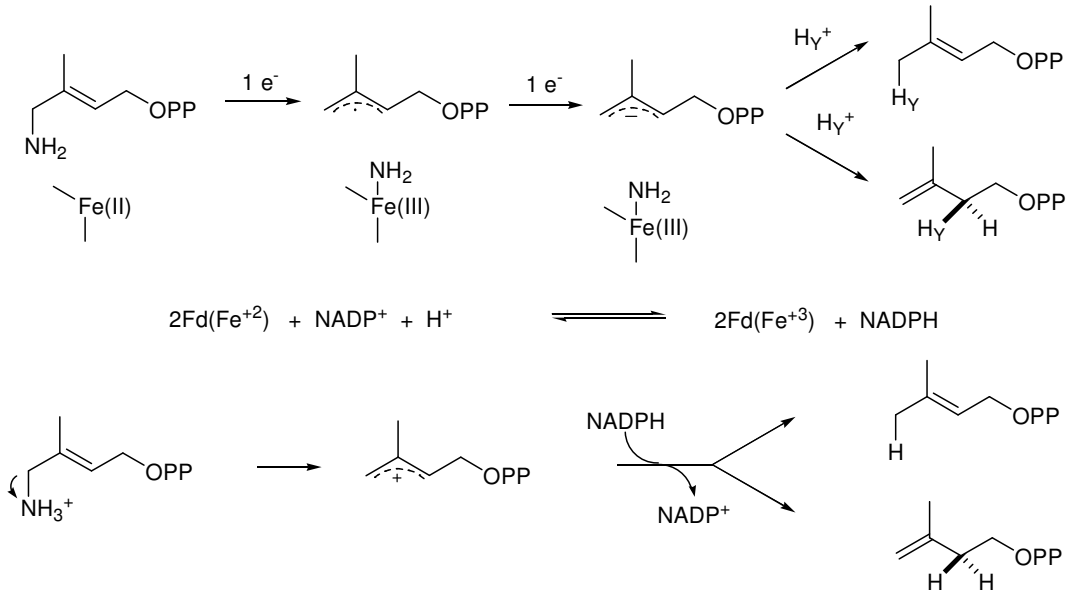
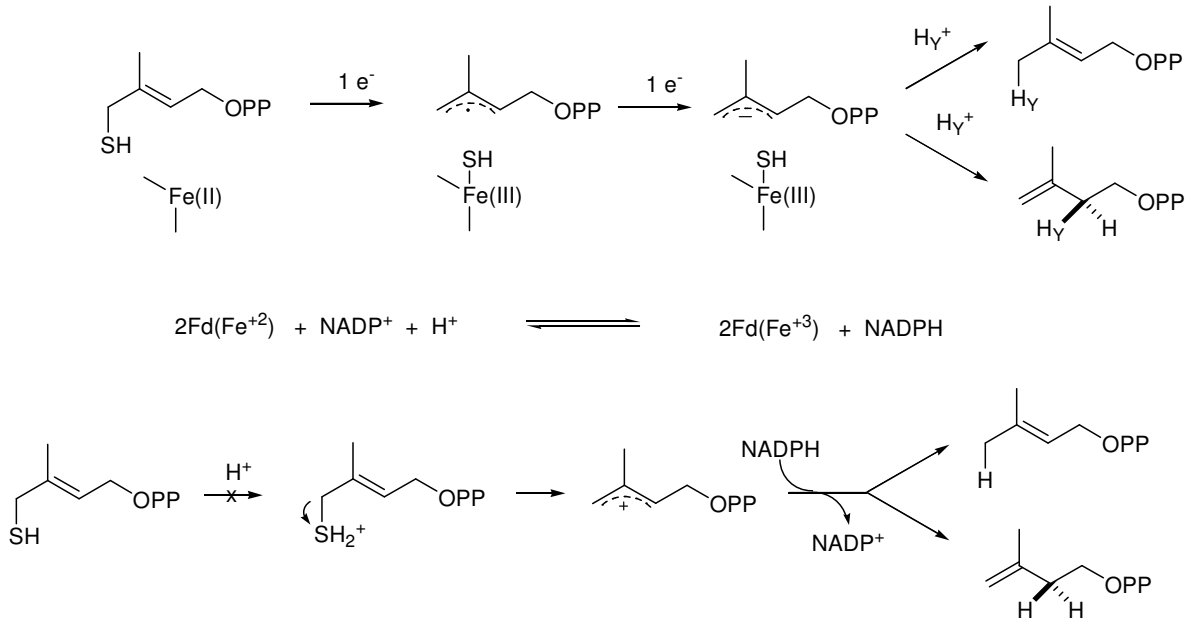


Figure 2.1. Proposed analogues for HDMAPP.

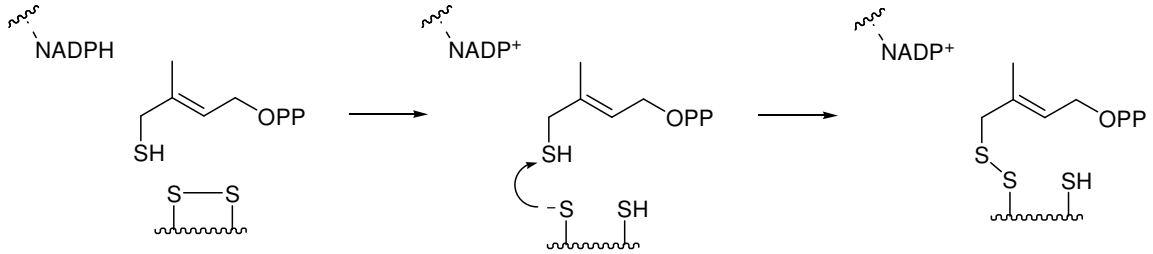


Scheme 2.6. Two hypothetical mechanisms for NDMAPP.

anion which is protonated to give either IPP or DMAPP. However, in the other mechanism, the protonation mechanism, SDMAPP will not be active (**Scheme 2.7**). Also, SDMAPP could inhibit the IspH by disrupting the iron-sulfur cluster (**Scheme 2.8**). The potential activity of the proposed analogues is shown in **Table 2.1**. NDMAPP is expected to be less active compared to HDMAPP in both mechanisms. On the other hand, the sulfur analogue should be more active than HDMAPP in the radical mechanism since the thiol group is a better ligand for Fe^{2+} than the hydroxyl group. However, the sulfur can not be protonated and will be less reactive in the protonation mechanism. Thus, these two analogues will help to distinguish the two mechanisms. Therefore, NDMAPP and SDMAPP could be potent inhibitors which would interact with the [4Fe-4S] cluster. Here we report the synthesis leading to these two analogues.



Scheme 2.7. Two hypothetical mechanisms for SDMAPP.



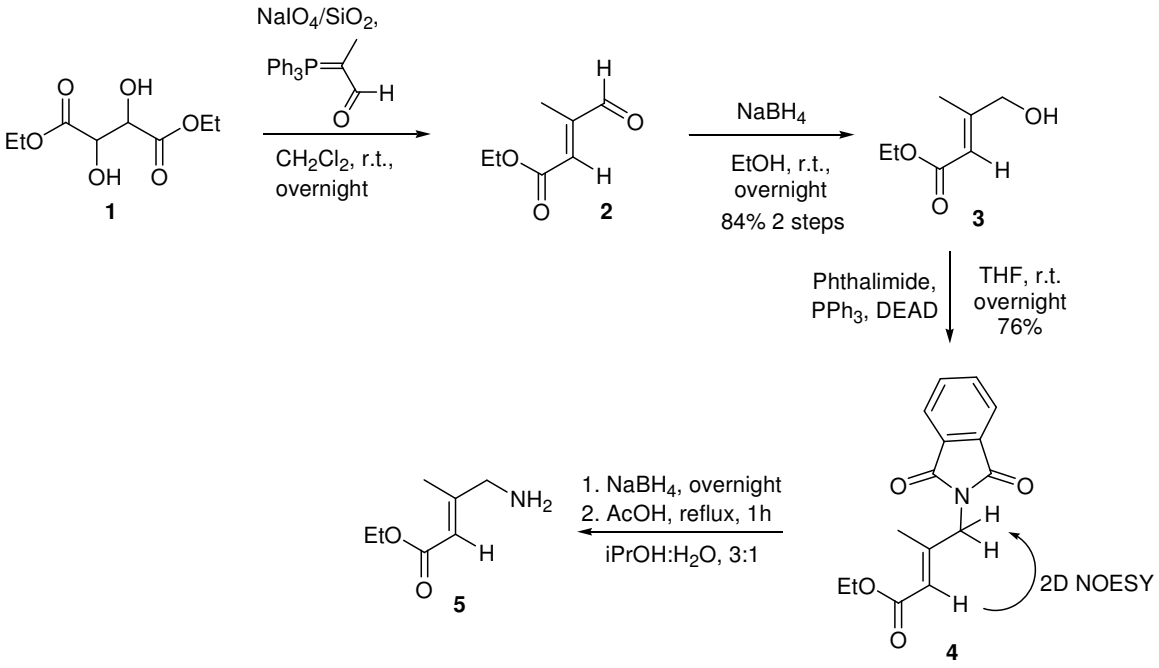
Scheme 2.8. Disruption of Fe-S cluster.

Table 2.1. The potential activity of NDMAPP and SDMAPP.

Bond type	Radical mechanism	Protonation mechanism
C-N	less active	less active
C-O	active	active
C-S	more active	less active

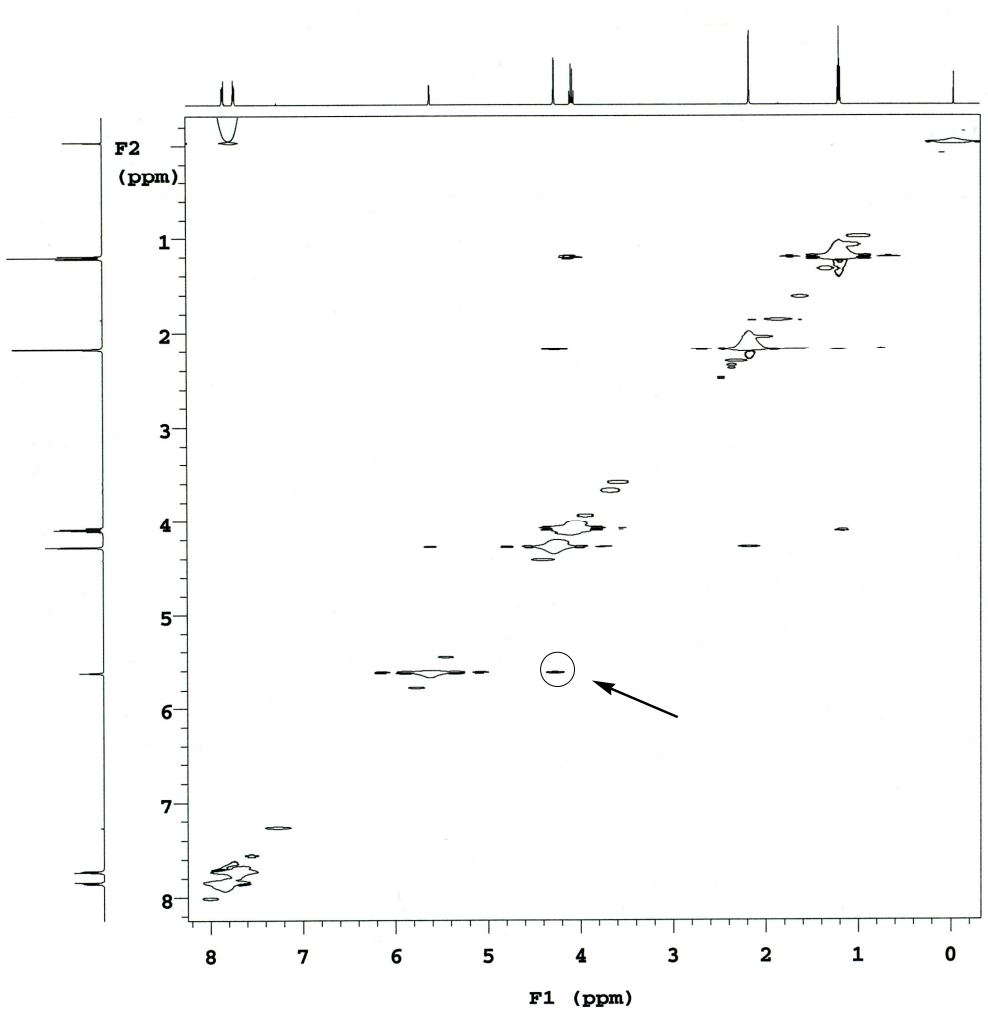
Synthesis of 4-Aminodimethylallyl Diphosphate

The synthesis of the amino analogue NDMAPP begins with the one-pot oxidative cleavage-Wittig sequence, which is a convenient way to prepare α,β-unsaturated esters.[19] Diethyl tartrate (**1**) is oxidized with $NaIO_4$ on silica gel (10% by weight)[20] to generate an aldehyde, which then reacts with the 2-(triphenylphosphoranylidene) ylid by Wittig reaction.[21] The resulting α,β-unsaturated aldehyde **2** was reduced with $NaBH_4$ without purification to give alcohol **3** in 84% in two steps. Allylic alcohol **3** was converted into phthalimide **4** using the Mitsunobu procedure[22] (**Scheme 2.9**). However, reduction of the phthalimide with $NaBH_4$, followed by acetic acid gave amine **5** in a low yield.[23] The stereochemistry of the double bond in phthalimide **4** was assigned by a NOESY experiment. The 2D NOESY spectrum of **4** showed a crosspeak between the olefinic proton at 5.64 ppm and the methylene protons at 4.31 ppm adjacent to the phthalimide moiety (**Figure 2.2**). This proved the expected *E* geometry of the double bond from the Wittig reaction. Alcohol **3** was protected with a TBDMS group and the resulting ester **6** was reduced with DIBALH to give alcohol **7**, which was then protected with a THP group to give **8**. The TBDMS group was removed with the Et_3N-3HF complex and hydroxy group of alcohol **9** was displaced by phthalimide under Mitsunobu conditions to give **10** (**Scheme 2.10**).[24] The amino group of **10** was unmasked with hydrazine, and the amine **11** was protected with Fmoc-OSu to give compound **12** (**Scheme 2.11**).[25] We chose to use this protecting group so the last deprotection step could be performed under basic conditions in order to preserve a diphosphate moiety of NDMAPP. The THP group of **12** was removed with PPTS in ethanol to give an alcohol **13**. Initially, we tried the



Scheme 2.9. Formation of amino ester **5**.

displacement methodology developed by Davisson *et al.* where allylic alcohol **13** was converted into allylic chloride **14** and then treated with tris(tetrabutylammonium) hydrogen diphosphate, to obtain diphosphate NDMAPP (**15**).[26] This method has the advantage that only one phosphate-containing compound is produced. Since the diphosphate salt is very basic, we thought that the Fmoc group might also be removed during phosphorylation to give NDMAPP (**Scheme 2.12**). However, the outcome was almost complete rearrangement of the starting material to alcohol **17** (**Scheme 2.13**). Under these conditions, we have two competitive reactions. The first reaction is a displacement of the chloride by inorganic diphosphate (path B), and the second is Fmoc protecting group cleavage, followed by rearrangement (path A). We speculate that the



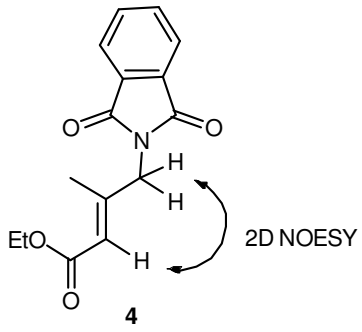
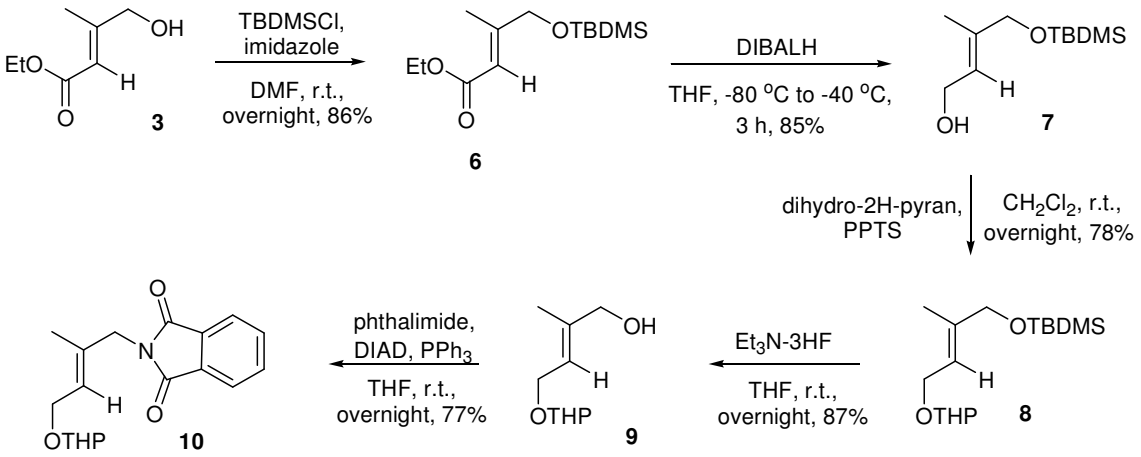
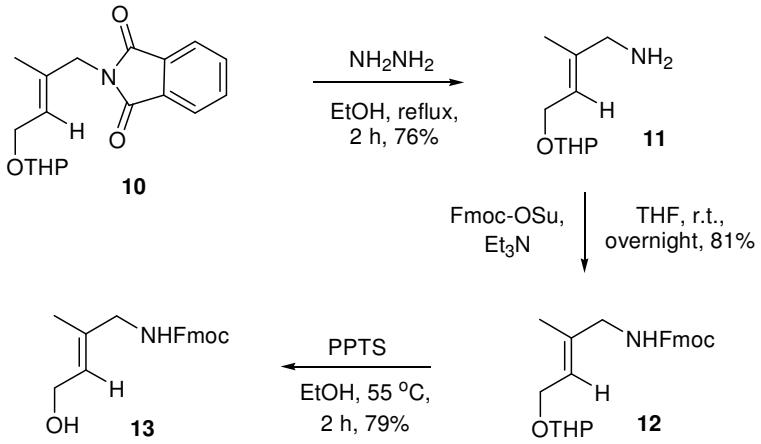


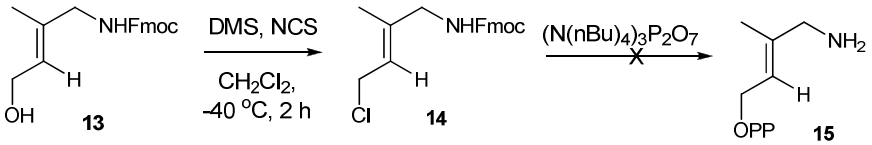
Figure 2.2. 2D NOESY experiment.



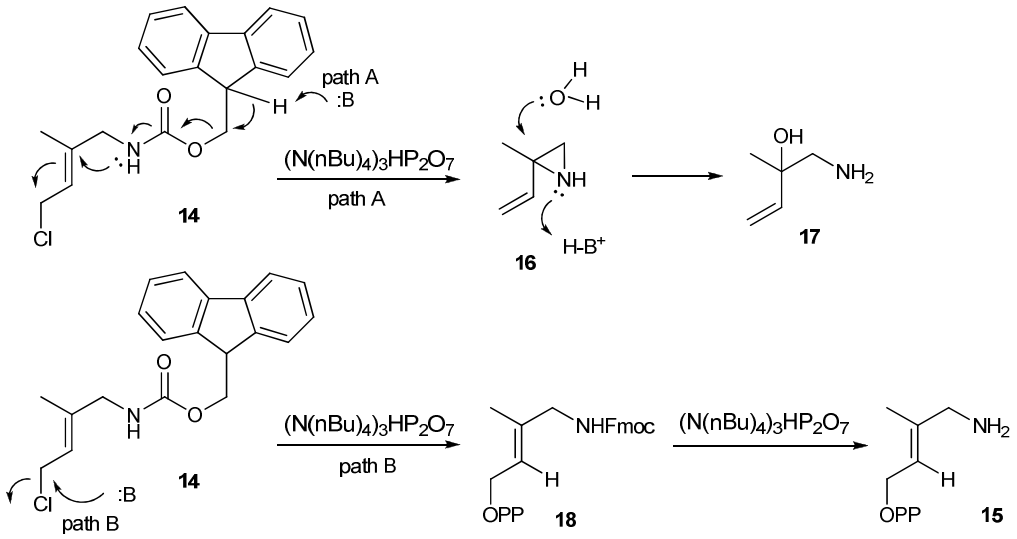
Scheme 2.10. Formation of phthalimide **10**.



Scheme 2.11. Synthesis of alcohol **13**.



Scheme 2.12. Synthesis of NDMAPP.

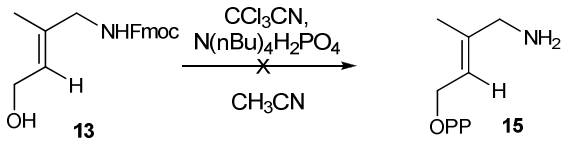


Scheme 2.13. Rearrangement under basic conditions.

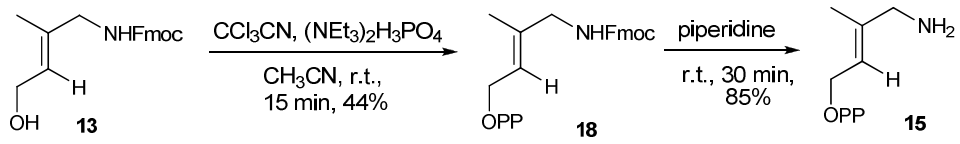
Fmoc deprotection is faster, followed by aziridine formation (**Scheme 2.13**). The aziridine **16** opening produces the tertiary alcohol **17**.

We then turned to the Cramer procedure.[27] The disadvantage of this method is formation of a mixture phosphates, mainly mono and diphosphates. We first tried the Danilov modification of the Cramer procedure using bis(tetrabutylammonium) phosphate and CCl_3CN (**Scheme 2.14**).[28] However, formation of the tertiary alcohol **17** was again observed. We then tried the original Cramer reaction modified by Keller and Thompson, using TEAP (bis(trimethyl ammonium) phosphate) solution and CCl_3CN.[29] Use of the TEAP eliminated the need for an ion-exchange step. The product is purified by chromatography on silica gel with elution by $iPrOH/H_2O/NH_4OH$ (**Scheme 2.15**).

The Fmoc group survived the purification step on silica using $iPrOH/H_2O/NH_4OH$ solvent mixture with pH = 12, and the Fmoc protected diphosphate **18** was obtained. The Fmoc group was removed with piperidine and NDMAPP (**15**) was obtained after purification on silica gel.[25]



Scheme 2.14. The Danilov modification.



Scheme 2.15. Formation of diphosphate **15**.

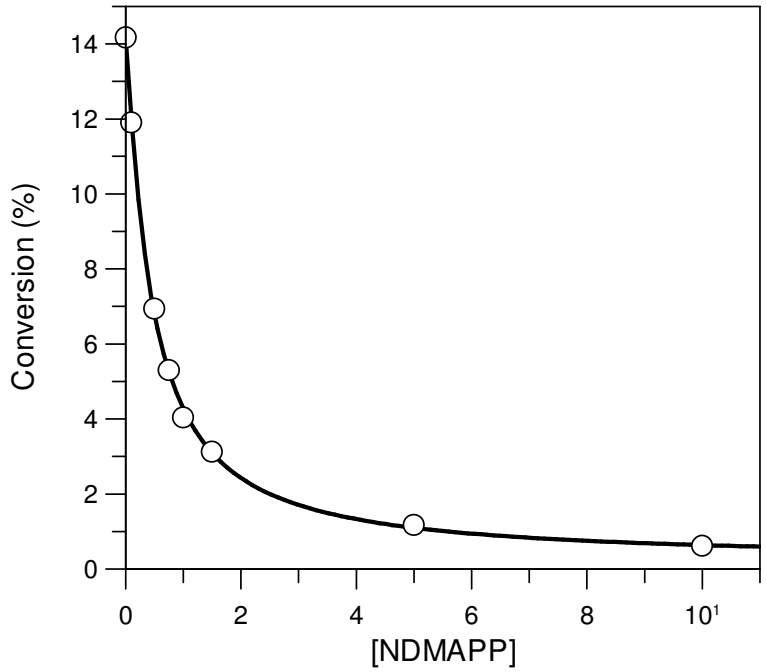
Enzymatic Evaluation of 4-Aminodimethylallyl Diphosphate

NDMAPP was examined as an inhibitor of IspG and IspH by Dr. Seemann in the laboratory of Professor Michel Rohmer at the University of Louis Pasteur, Institut de Chimie, Strasbourg, France. NDMAPP did not inhibit IspG at concentrations 10 μM, 100 μM and 1 mM. The experimental results with IspH are shown in **Table 2.2**. The IC_{50} value 0.45 μM was determined using GraFit™ software (see **Figure 2.3**). At this point, we cannot determine the mode of action of this analogue. It will be important to see if this substrate is a reversible or a time-dependent irreversible inhibitor of IspH.

The NDMAPP analogue was also tested against type I and II IDI isomerase enzymes. The radioactive assay showed no inhibition activity of NDMAPP against IDI II. However, NDMAPP was active against IDI I (**Table 2.3**) with an IC_{50} value 21 μM (**Figure 2.4**).

Table 2.2. IC_{50} determination for the NDMAPP analogue with IspH.

Inhibitor concentration (µM)	Specific activity (µmole/min/mg)	Conversion (%)	Residual activity (%)	Log [I]
0,000	0,1446442	14,17	100,000	-
0,100	0,1214711	11,90	83,979	-7,000
0,500	0,0708923	6,94	49,012	-6,301
0,750	0,0541032	5,30	37,404	-6,125
1,000	0,0412627	4,04	28,527	-6,000
1,500	0,0318190	3,12	21,998	-5,824
5,000	0,0119782	1,17	8,281	-5,301
10,000	0,0062211	0,61	4,301	-5,000

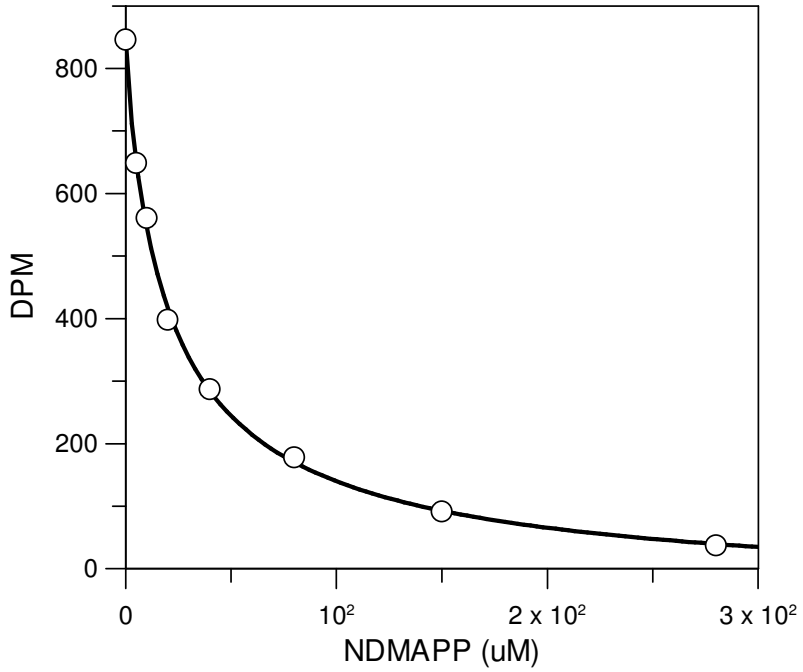


Parameter	Value	Std. Error
Y Range	13.9193	0.2461
IC 50	0.4521	0.0176
Slope factor	1.1223	0.0494
Background	0.2191	0.1673

Figure 2.3. IC_{50} determination for the NDMAPP analogue with IspH.

Table 2.3. IC_{50} determination for the NDMAPP analogue with IDI type I.

Conc. Of NDMAPP	Radioactivity trial 1	trial 2	Average	Without background
0	823	1027	925	846
5	703	753	728	649
10	656	624	640	561
20	478	476	477	398
40	403	329	366	287
80	257	257	257	178
150	172	168	170	91
280	118	114	116	37
No enzyme	79	79		
1/2 buffer	18499	18499		

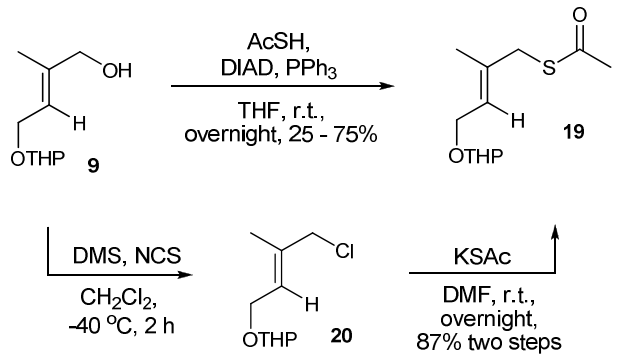


Parameter	Value	Std. Error
Y Range	894.5310	33.3710
IC 50	21.8322	2.1627
Slope factor	0.8712	0.0610
Background	-48.0494	28.6495

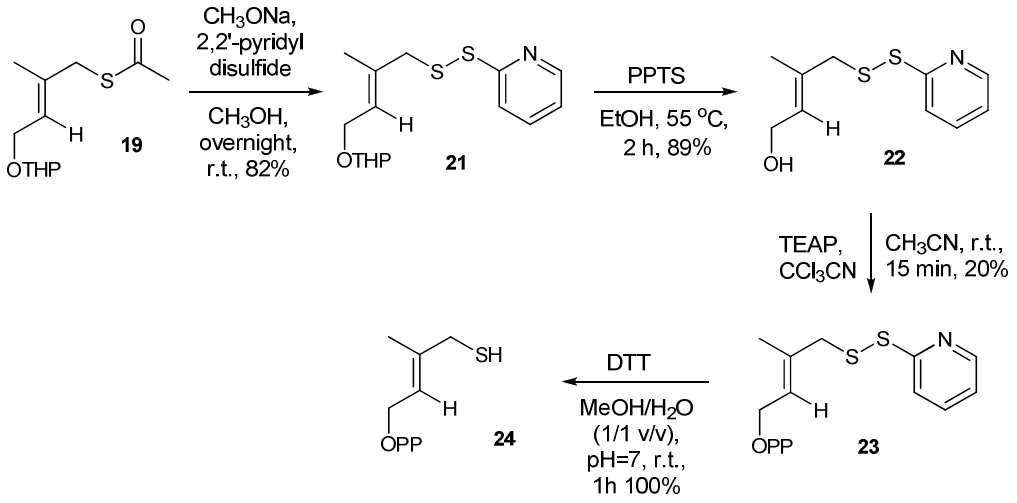
Figure 2.4. IC_{50} determination for the NDMAPP analogue with IDI type I.

Synthesis of 4-Thiodimethylallyl Diphosphate

The first step in the synthesis of SDMAPP is shown in **Scheme 2.16**. The hydroxy group of alcohol **9** was esterified to give thioacetate **19**. Initially, we tried a Mitsunobu displacement with thioacetic acid.[30] However, the reaction was not reproducible, and the yields varied from 25 to 75%. We found that generally, a better yield was obtained when thioacetic acid added dropwise to the solution of the PPh_3, DIAD, and corresponding alcohol. The thioacetate **19** was prepared by conversion of alcohol **9** into corresponding allylic chloride **20**,[31] followed by displacement of chloride with potassium thioacetate to give **19** (**Scheme 2.16**). The acetate group of **19** was removed with CH_3ONa at -40 ^oC (**Scheme 2.17**). The free thiol group was susceptible to oxidation to form a symmetric disulfide in SDMAPP and the intermediates leading to the analogue. When left unprotected under mild basic conditions (pH = 7.2), 20% conversion of SDMAPP to symmetric disulfide is observed within 1 hour. We chose 2,2'-pyridyl disulfide, which can be easily removed in the presence of diphosphate in aqueous media immediately before SDMAPP is used in experiments. This reagent is widely used to protect cysteine residues in peptide synthesis.[32]



Scheme 2.16. Preparation of thioacetate **19**.



Scheme 2.17. Synthesis of SDMAPP**.**

The mixed pyridyl disulfide is stable to acid/base conditions, and only sensitive to reducing agents. Therefore, the liberated thiol of **19** was treated with 2,2'-pyridyl disulfide to give mixed 2-pyridyl disulfide **21**. The THP group was then removed to give alcohol **22**. We tried to introduce the diphosphate using the procedure of Davisson *et al.*[26] however, the allylic chloride could not be prepared from alcohol **22**. Instead, the alcohol **22** was phosphorylated to give disulfide **23** using the Cramer procedure.[29] To prevent formation of symmetric disulfide, the SDMAPP is stored as asymmetric disulfide **23** at -80 ^{o}C until needed (**Scheme 2.17**). The disulfide **23** is treated with DTT for 1 hour to give the free thiol SDMAPP (**24**) in 90% yield. This reaction can be performed directly in the assay buffer, since DTT is usually added to it to preserve a protein.

In summary, the synthesis of NDMAPP and SDMAPP analogues of HDMAPP were achieved. The NDMAPP was found to be inactive against IspG and IDI II protein. It was active against IspH protein with IC_{50} = 0.45 μM, as well as IDI. This molecule

will be evaluated as an inhibitor or substrate of IspG and IspH by our coworkers in France. The studies are underway and will be reported separately.

Experimental Section

General methods

NMR spectra were recorded on a Varian Unity Inova NMR 300 MHz FTNMR interfaced to a Sun Sparcstation 5 computer at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR). All chemical shifts are reported in parts per million (ppm) and coupling constants (J) in hertz (Hz). ^{1}H chemical shifts are referenced relative to the TMS peak at δ 0.0 ppm or to the peak of CD_2Cl_2 at 5.32 ppm. The ^{13}C chemical shifts are referenced relative to $CDCl_3$ at δ 77.0 ppm (center peak), the peak of CD_2Cl_2 at 54 ppm (center peak), and for D_2O to MeOH at 49 ppm. ^{31}P chemical shifts are referenced relative to the peak of H_3PO_4 in D_2O at δ 0 ppm.

All commercial reagents were ACS reagent grade and were used without further purification. In general all solvents, reagents, and deuterated solvents were purchased from Fisher Chemicals, Acros, and Aldrich. Methylene chloride and ethanol were distilled from CaH_2 prior to use. All other reagents were of commercial quality from freshly opened containers.

General procedure for THP deprotection (13, 22). PPTS (25 mg, 0.1 mmol) was added to the solution of THP protected alcohol (1 mmol) in EtOH (10 mL). The resulted solution was stirred at 55 ^{o}C for 1 h. Then solution was cooled down to room temperature. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate.

General procedure for phthalimide coupling (4, 10). A solution of DIAD (223 mg, 1.1 mmol) in THF (1 mL) was added dropwise via a syringe to a solution of an alcohol (1 mmol), phthalimide (162 mg, 1.1 mmol), and PPh_3 (289 mg, 1.1 mmol), in THF (4 mL) at the room temperature. After stirring overnight the solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate.

Cramer procedure for phosphorylation (18, 23). Phosphorylations were performed on 200 µmol scale according to the procedure of Keller and Thompson.[29] To the alcohol (200 µmol) in CH_3CN (2 mL) was added CCl_3CN (0.5 mL, 5 mmol), and TEAP solution was added in three portions after a 5-min period (3x0.5 mL). The TEAP solution was prepared by slowly mixing 9.1 mL of solution A and 1.5 mL of solution B at 0 ^oC. Solution A consisted of concentrated phosphoric acid (2.5 mL) in CH_3CN (9.4 mL), and solution B of triethyl amine (11 mL) in CH_3CN (10 mL). The reaction mixture was allowed to stir for 0.5 h at room temperature. Solvents were removed at reduced pressure, and the residue was chromatographed on silica with elution by iPrOH:H_2O:NH_4OH (6:0.5:2.5 v/v/v). The fractions were analyzed by silica TLC in iPrOH:H_2O:NH_4OH (6:1:3 v/v/v) and visualized by *p*-anisaldehyde. The fractions containing the product were concentrated under reduced pressure, and lyophilized.

Preparation of $NaIO_4$ on SiO_2. $NaIO_4$ (2 g) was dissolved in 10 mL of deionized H_2O at 70 ^oC. The solution was then added to a suspension of SiO_2 (20 g) in 40 mL of deionized H_2O. The mixture was stirred at 20 ^oC for 30 min. H_2O was removed using a rotary evaporator. Two portions of benzene (50 mL) were added and evaporated similarly. The reagent was dried in vacuo using an oil pump with gentle heating for 3 h.

(*E*)-4-Hydroxy-*E*-3-methyl-but-2-enoic acid ethyl ester (3). To a 250 mL round bottom flask was loaded $NaIO_4$ on SiO_2 (100.74 g, 47.1 mmol, 10% by weight), L-(+)-diethyl tartrate (3.24 g, 15.7 mmol) and 2-(triphenylphosphoranylidene) propionaldehyde ylid (5 g, 15.7 mmol) in CH_2Cl_2 (200 mL). After stirring overnight, the solution was filtered. The white solid was washed with 200 mL of ether, and the combined organic layers were concentrated under reduced pressure. The residue was redissolved in EtOH. To the resulting solution was added $CeCl_3$ heptahydrate (17.57 g, 47.1 mmol) and when the reagent had dissolved $NaBH_4$ (1.78 g, 47.1 mmol) was added in small portions during 15 min. The resulted mixture was stirred overnight. The pH of the solution was then adjusted to 7 using 1M HCl, and the solvent was removed at reduced pressure. The aqueous layer was saturated with NaCl and extracted with ether (3x50 mL). The ether layers were combined, washed with saturated Na_2CO_3 and brine, and dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate to give 1.92 g (84%) of product **3** as colorless oil. R_f 0.39 (hexanes:ethyl acetate 2:3); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.28 (3H, t, J = 8.0 Hz, CH_3), 2.09 (3H, t, J = 0.6 Hz, CH_3), 2.30 (1H, s, OH), 4.13 (2H, s, CH_2), 4.17 (2H, q, J = 8.0 Hz, CH_2) 5.98 (1H, m, J = 1.7 Hz, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 13.95, 15.28, 59.58, 66.38, 113.07, 157.73, 167.02; **HRMS** (EI) calcd. for $C_7H_{12}O_3$ 144.0786, found 144.0789.

(*E*)-4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-3-methyl-but-2-enoic acid ethyl ester (4). Alcohol **3** (100 mg, 0.7 mmol) was converted into phthalimide **4** using the general procedure described above. The residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate to give 144 mg (76%) of product **4** as white

solid. R_f 0.36 (hexanes:ethyl acetate 3:2); mp=128-129 ^oC (EtOH); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.24 (3H, s, J = 8.0 Hz, CH_3), 2.22 (3H, t, J = 0.7 Hz, CH_3), 4.13 (2H, q, J = 8.0 Hz, CH_2), 4.31 (2H, d, J = 0.7 Hz, CH_2), 5.64 (1H, m, J = 1.7 Hz, CH), 7.46 (2H, d, J = 5.6 Hz, 2CH), 7.81 (2H, d, J = 5.6 Hz, 2CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 14.22, 16.88, 44.45, 59.89, 115.97, 123.59, 131.84, 134.27, 151.67, 166.00, 167.75; **HRMS** (CI) calcd. for $C_{15}H_{16}NO_4$ [M+H$^+$] 274.1079, found 274.1097.

(*E*)-4-(*tert*-Butyl-dimethyl-silanyloxy)-3-methyl-but-2-enoic acid ethyl ester (6). Under nitrogen atmosphere, TBDMSCl (2.88 g, 19.10 mmol) in DMF (20 mL) was added dropwise into solution of alcohol **3** (2.5 g, 17.36 mmol) and imidazole (1.77 g, 26.04 mmol) in DMF (20 mL). The resulted mixture was stirred overnight at room temperature. The progress of the reaction was followed by TLC. The solution was transferred into a separation funnel containing 500 mL of water, extracted with Et_2O (3x50 mL) and the combined extracts were dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate to give 4.5 g (86%) of product **6** as colorless oil. R_f 0.67 (hexanes:ethyl acetate 1:1); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 0.07 (6H, s, $2CH_3$), 0.90 (9H, s, $3CH_3$), 1.17 (3H, s, CH_3), 1.30 (3H, t, J_1 = 8 Hz, CH_3), 4.24 (2H, m, CH_2), 3.57 (2H, dd, J_1 = 47 Hz, J_2 = 11 Hz, CH_2), 5.32 (1H, t, J = 1.Hz, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ -5.47, 14.34, 15.42, 18,35, 25.86, 59.57, 67.08, 113.32, 157.16, 167.10; **HRMS** (FTMS) calcd. for $C_{13}H_{27}O_3Si$ [M+H$^+$] 259.1729, found 259.1724.

(*E*)-4-(*tert*-Butyl-dimethyl-silanyloxy)-3-methyl-but-2-en-1-ol (7). Under nitrogen atmosphere, a solution of 1M DIBALH in hexanes (62 mL, 62 mmol) was added dropwise via a syringe to a solution of ester **6** (4 g, 15.5 mmol) in THF (60 mL) at –78

^{0}C. The mixture was stirred for 5 h. The temperature was allowed to rise to –40 ^{0}C. After the addition of brine (20 mL), the mixture was stirred for 30 min at room temperature. The solution was filtered through celite®, and the filter cake was washed with ether (3x20 mL). NaCl was added until the phases separated. The organic layer was removed, and the aqueous layer was extracted with ether (3x30 mL). The organic layers were combined, and dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate to give 2.85 g (85%) of product **7** as colorless oil. R_f 0.36 (hexanes:ethyl acetate 3:2); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 0.08 (6H, s, $2CH_3$), 0.92 (9H, s, $3CH_3$), 1.25 (1H, t, J = 6 Hz, OH), 1.65 (3H, t, J = 0.6 Hz, CH_3), 4.03 (2H, s, CH_2), 4.21 (2H, t, J = 6 Hz, CH_2), 5.68 (1H, m, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ -5.37, 13.47, 18.38, 25.90, 59.09, 67.62, 122.51, 138.25; **HRMS** (FTMS) calcd. for $C_{11}H_{25}O_2Si$ $[M+H^+]$217.1624, found 217.1619.

(*E*)-*tert*-Butyl-dimethyl-[2-methyl-4-(tetrahydro-pyran-2-yloxy)-but-2-enyloxy]-silane (8). To alcohol **7** (2.5 g, 11.55 mmol) in CH_2Cl_2 (60 mL) was added 3,4-dihydro-2H-pyran (1.46 g, 17.33mmol) in CH_2Cl_2 (10 mL), and PPTS (289 mg, 1.16 mmol) in CH_2Cl_2 (1 mL). After stirring overnight, the solution was washed with saturated $NaHCO_3$ (10 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3x20 mL). The combined organic extracts were dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate to give 2.7 g (78%) of product **8** as colorless oil. R_f 0.78 (hexanes:ethyl acetate 1:1); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 0.07 (6H, s, $2CH_3$), 0.91 (9H, s, $3CH_3$), 1.65 (9H, m, CH_3, $3CH_2$), 3.51 (1H, m, CH), 3.90 (1H, m, CH), 4.04 (2H, s, CH_2), 4.1 (1H, dd, J_1 = 12 Hz, J_2 = 5 Hz, CH), 4.27 (1H, dd, J_1 = 12 Hz, J_2 = 5 Hz,

CH), 4.63 (1H, m, CH), 5.63 (1H, m, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ -5.34, 13.61, 18.39, 19.55, 25.49, 25.92, 30.67, 62.26, 63.16, 67.88, 97.76, 120.18, 138.89; **HRMS** (MALDI) calcd. for $C_{16}H_{32}O_3SiNa$ [M+Na^+] 323.2013, found 323.2005.

(*E*)-2-Methyl-4-(tetrahydro-pyran-2-yloxy)-but-2-en-1-ol (9). Under nitrogen atmosphere, Et_3N-3HF complex (10.8 g, 67 mmol) in THF (20 mL) was added dropwise to the solution of corresponding protected alcohol **8** (2 g, 6.7 mmol) in THF (20 mL). After stirring overnight, Et_3N (10 mL) was added. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate to give 1.1 g (87%) of product **9** as colorless oil. R_f 0.36 (hexanes:ethyl acetate 1:1); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.71 (10H, m, CH_3, $3CH_2$, OH), 3.53 (1H, m, CH), 3.89 (1H, m, CH), 4.04 (2H, d, J = 0.6 Hz, CH_2), 4.07 (1H, dd, J_1 = 12 Hz, J_2 = 5 Hz, CH), 4.30 (1H, dd, J_1 = 12 Hz, J_2 = 5 Hz, CH), 4.65 (1H, m, CH), 5.64 (1H, m, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 13.77, 19.44, 25.37, 30.57, 62.21, 63.22, 67.96, 98.01, 121.13, 139.12; **HRMS** (MALDI) calcd. for $C_{10}H_{18}O_3Na$ [M+Na^+] 209.1148, found 209.1150.

(*E*)-2-[2-Methyl-4-(tetrahydro-pyran-2-yloxy)-but-2-enyl]-isoindole-1,3-dione (10). Alcohol **9** (850 mg, 4.5 mmol) was converted into phthalimide **10** using the general procedure described above. The residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate to give 1.1 g (77%) of product **10** as white solid. mp=58-59 ^{o}C; R_f 0.66 (hexanes:ethyl acetate 1:1); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.64 (9H, m, CH_3, $3CH_2$), 3.48 (1H, m, CH), 3.84 (1H, m, CH), 4.05 (1H, m, CH), 4.23 (3H, m, CH, CH_2), 4.59 (1H, m, CH), 5.52 (1H, m, CH), 7.74 (2H, m, 2CH), 7.85 (2H, m, 2CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 14.95, 19.40, 25.40, 30.54, 44.45, 62.12, 63.11, 97.75, 123.32,

123.56, 132.01, 133.34, 133.98, 134.28, 168.10; **HRMS** (MALDI) calcd. for $C_{18}H_{21}NO_4Na$ [M+Na^+] 338.1363, found 338.1368.

(*E*)-2-Methyl-4-(tetrahydro-pyran-2-yloxy)-but-2-enylamine (11). To a solution of phthalimide **10** (445 mg, 1.4 mmol) in EtOH (10 mL) was added hydrazine hydrate (210 mg, 4.2 mmol) in EtOH (2 mL). The solution was warmed to 50 ^{0}C and stirred at that temperature for 30 min, followed by refluxing for 2 h. The solution was allowed to cool and filtered. The filter cake was washed with EtOH (3x10 mL). The filtrates were combined and solvent was removed at reduced pressure. Ether (20 mL) and 1M NaOH were added (5 mL) to adjust the pH to 12. The aqueous layer was saturated with NaCl and extracted with ether (3x20 mL). The combined organic extracts were dried over Na_2SO_4. The solvent was removed at reduced pressure to give 1.1 g (54%) of product **11** as yellow oil, which was used without further purification.

(*E*)-[2-Methyl-4-(tetrahydro-pyran-2-yloxy)-but-2-enyl]-carbamic acid 9*H*-fluoren-9-ylmethyl ester (12). To a solution of amino ester **11** (480 mg, 2.6 mmol) in THF (10 mL) were added in sequence triethyl amine (290 mg, 2.85 mmol) in THF (6 mL), and *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (1.3 g, 3.9 mmol) in THF (10 mL). After stirring overnight solvent was removed at reduced pressure, and the residue was purified by chromatography using a silica column, which was eluted with hexanes/ethyl acetate by gradient method to give 850 mg (81%) of product **12** as white solid. mp=126-127 ^{o}C; R_f 0.36 (hexanes:ethyl acetate 3:2); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.68 (9H, m, CH_3, $3CH_2$), 3.52 (1H, m, CH), 3.77 (2H, d, J = 6 Hz, CH_2), 3.88 (1H, m, CH), 4.03 (1H, dd, J_1 = 12 Hz, J_2 = 5 Hz, CH), 4.25 (2H, m, 2CH), 4.42 (2H, d, J = 3.4 Hz, CH_2), 4.62 (1H, m, CH), 4.86 (1H, s broad, 1NH), 5.52 (1H, m, CH), 7.31 (2H, dt, J_1

= 7.5 Hz, J_2 = 1.2 Hz, 2CH), 7.40 (2H, t, J = 7.5 Hz, 2CH), 7.59 (2H, d, J_1 = 7.5 Hz, 2CH), 7.76 (2H, d, J = 7.5 Hz, 2CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 14.65, 19.53, 25.40, 30.62, 47.25, 47.93, 62.32, 63.34, 66.65, 98.19, 119.95, 121.99, 125.01, 127.01, 127.65, 136.36, 141.29, 143.91, 156.38; **HRMS** (MALDI) calcd. for $C_{25}H_{29}NO_4Na$ [M+Na^+] 430.1989, found 430.1983.

(*E*)-(4-Hydroxy-2-methyl-but-2-enyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (13). An acetal **12** (850 mg, 2.08 mmol) was deprotected following the general procedure for THP deprotection described above. The residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate to give 532 mg (79%) of product **13** as white solid. mp=131-133 ^oC; R_f 0.21 (hexanes:ethyl acetate 2:3); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.4 (1H, s broad, OH), 1.67 (3H, s, CH_3), 3.74 (2H, d, J = 7 Hz, CH_2), 4.21 (3H, m, CH_2, CH), 4.44 (2H, d, J = 7 Hz, CH_2), 4.91 (1H, s broad, 1NH), 5.52 (1H, m, CH), 7.32 (2H, dt, J_1 = 7.5 Hz, J_2 = 1.2 Hz, 2CH), 7.40 (2H, m, 2CH), 7.59 (2H, d, J_1 = 7.5 Hz, 2CH), 7.77 (2H, d, J = 7.5 Hz, 2CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 14.51, 47.24, 47.77, 58.95, 66.57, 119.96, 124.33, 124.96, 127.01, 127.65, 135.59, 141.29, 143.86, 156.43; **HRMS** (MALDI) calcd. for $C_{20}H_{21}NO_3Na$ [M+Na^+] 346.1414, found 346.1427.

(*E*)-(2-Methyl-but-2-enyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester 4-diphosphate bis ammonium salt (18). An alcohol **13** (65 mg, 20 μmol) was phosphorylated according to the Cramer procedure described earlier. The residue was chromatographed on silica with elution by iPrOH:H_2O:NH_4OH (6:0.5:2.5 v/v/v). The fractions were analyzed by silica TLC (iPrOH:H_2O:NH_4OH (6:1:3 v/v/v)) and visualized by *p*-anisaldehyde. The fractions containing the product were concentrated under reduced pressure, and then lyophilized to give 46 mg (44%) of product **18** as white solid. R_f 0.30

(iPrOH:H_2O:NH_4OH (6:1:3 v/v/v)); **^{1}H NMR** (D_2O, 300 MHz) δ 1.53 (3H, s, CH_3), 3.52 (2H, s, CH_2), 4.25 (1H, m, CH), 4.43 (2H, t, J = 6.3 Hz, CH_2), 4.55 (2H, d, J = 4.8 Hz, CH_2), 5.34 (1H, m, CH), 7.36 - 7.49 (4H, m, 4CH), 7.65 (2H, d, J_1 = 7.5 Hz, 2CH), 7.87 (2H, d, J = 7.5 Hz, 2CH); **^{13}C NMR** (D_2O, 75 MHz) δ 13.77, 23.76, 47.30, 62.08, 66.03, 81.71, 120.24, 125.07, 127.58, 128.14, 137.61, 141.10, 143.93, 158.45; **^{31}P NMR** (D_2O, 125 MHz) δ -9.73 (d, J = 22 Hz), -6.92 (d, J = 22 Hz); **HRMS** (MALDI) calcd. for $C_{20}H_{22}NO_9P_2$ [M-H] 482.0775, found 482.0759.

(*E*)-4-Amino-3-methylbut-2-enyl diphosphate bis ammonium salt (15). A diphosphate **18** (120 mg, 0.23 μmol) was added to piperidine (5 mL) and stirred for 1 h at room temperature. The piperidine was removed, and the residue was purified on silica with elution by iPrOH:H_2O:NH_4OH (6:0.5:2.5 v/v/v). The fractions were analyzed by silica TLC (iPrOH:H_2O:NH_4OH (6:1:3 v/v/v)) and visualized by *p*-anisaldehyde. The fractions containing the product were concentrated under reduced pressure, and then lyophilized to give 58 mg (85%) of product **15** as white solid. R_f 0.1 (iPrOH:H_2O:NH_4OH (6:1:3 v/v/v)); **^{1}H NMR** (D_2O, 300 MHz) δ 1.53 (3H, s, CH_3), 3.07 (2H, s, CH_2), 4.32 (2H, t, J = 8 Hz, CH_2), 5.41 (1H, m, CH); **^{13}C NMR** (D_2O, 75 MHz) δ 14.36, 45.51, 62.14, 62.20, 125.58, 131.94; **^{31}P NMR** (D_2O, 125 MHz) δ -9.73 (d, J = 22 Hz), -6.92 (d, J = 22 Hz); **HRMS** (MALDI) calcd. for $C_5H_{12}NO_7P_2$ [M-H] 260.0095, found 260.0092.

(*E*)-Thioacetic acid S-[2-methyl-4-(tetrahydro-pyran-2-yloxy)-but-2-enyl] ester (19). To NCS (176 mg, 1.29 mmol) in CH_2CL_2 (4 mL) at -40 ^oC was added DMS (103 μL, 1.39 mmol) in CH_2CL_2 (3 mL). The mixture was stirred for 10 min at 0 ^oC, cooled down to -40 ^oC, and alcohol **9** (200 mg, 1.07 mmol) in CH_2CL_2 (3 mL) was added

dropwise. The mixture was stirred for 2 h at 0 ^{o}C and periodically monitored by silica TLC. When the starting material had disappeared, the reaction was quenched with brine (10 mL). The layers were separated, and the aqueous layer was extracted with CH_2CL_2 (3x10 mL). Organic layers were combined and dried over Na_2SO_4. The residue was concentrated under reduced pressure and redissolved in DMF (2 mL). KSAc (499 mg, 4.28 mmol) in DMF (8 mL) was added at room temperature, and the solution was stirred overnight. H_2O (100 mL) was added and the product was extracted with ether (3x20 mL). The organic layer was washed with saturated $NaHCO_3$ (4x30 mL) and brine (30 mL). Organic layers were combined and dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate to give 228 mg (87%) of product **19** as colorless oil. R_f 0.51 (hexanes:ethyl acetate 1:1); **1H NMR** ($CDCl_3$, 300 MHz) δ 1.67 (9H, m, CH_3, $3CH_2$), 2.35 (3H, s, CH_3), 3.51 (1H, m, CH), 3.58 (1H, m, CH), 3.87 (2H, s, CH_2), 4.02 (1H, dd, J_1 = 12 Hz, J_2 = 5 Hz, CH), 4.23 (1H, dd, J_1 = 12 Hz, J_2 = 5 Hz, CH), 4.61 (1H, m, CH), 5.62 (1H, m, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 15.51, 19.48, 25.43, 30.47, 30.60, 37.68, 62.24, 63.44, 97.96, 124.98, 134.81; **HRMS** (MALDI) calcd. for $C_{12}H_{20}O_3SNa$ [M+Na^+] 267.1025, found 267.1026.

(*E*)-2-[2-Methyl-4-(tetrahydro-pyran-2-yloxy)-but-2-enyldisulfanyl]-pyridine (21). To a solution of thioacetate **19** (245 mg, 1 mmol) and 2,2'-pyridine disulfide (661 mg, 3 mmol) in CH_3OH (10 mL) was added 30% wt solution of CH_3ONa in CH_3OH (180 mg, 1 mmol) at -40 ^{o}C. The mixture was left in a freezer at -20 ^{o}C overnight without stirring. The solution was concentrated under reduced pressure, and the residue was purified by chromatographed on silica with gradient elution by hexanes/ethyl acetate to

give 256 mg (82%) of product **21** as colorless oil. R_f 0.38 (hexanes:ethyl acetate 3:2); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.68 (9H, m, CH_3, $3CH_2$), 3.41 (2H, s, CH_2), 3.49 (1H, m, CH), 3.84 (1H, m, CH), 3.94 (1H, dd, J_1 = 12 Hz, J_2 = 5 Hz, CH), 4.13 (1H, dd, J_1 = 12 Hz, J_2 = 5 Hz, CH), 4.57 (1H, m, CH), 5.50 (1H, m, CH), 7.07 (1H, m, CH), 7.63 (2H, m, 2CH), 8.45 (1H, m, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 15.55, 19.45, 25.41, 30.59, 48.23, 62.21, 63.32, 97.80, 119.68, 120.50, 127.00, 133.41, 136.76, 149.56, 160.24; **HRMS** (MALDI) calcd. for $C_{15}H_{22}NO_2S_2$ [M+H$^+$] 312.1086, found 312.1097.

***(E)*-3-Methyl-4-(pyridin-2-yldisulfanyl)-but-2-en-1-ol (22).** Acetal **21** (830 mg, 2.6 mmol) was deprotected following the general procedure for THP deprotection described above. The residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate to give 540 mg (89%) of product **22** as colorless oil. R_f 0.43 (hexanes:ethyl acetate 1:1); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.56 (1H, t, J = 6 Hz, OH), 1.69 (3H, s, CH_3), 3.37 (2H, d, J = 1 Hz, CH_2), 4.03 (2H, d, J = 6 Hz, CH_2), 5.53 (1H, m, CH), 7.09 (1H, m, CH), 7.65 (2H, m, 2CH), 8.45 (1H, m, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 15.21, 47.97, 59.11, 120.80, 120.86, 129.98, 132.58, 136.92, 149.36, 160.53; **HRMS** (MALDI) calcd. for $C_{10}H_{14}NOS_2$ [M+H$^+$] 228.0511, found 228.0521.

(*E*)-[3-Methyl-4-(pyridin-2-yldisulfanyl)-but-2-enyl] diphosphate ammonium salt (23). An alcohol **22** (46 mg, 20 μmol) was phosphorylated according to the Cramer procedure described earlier. The residue was chromatographed on silica eluted by iPrOH:H_2O:NH_4OH (6:0.5:2.5 v/v/v). The fractions were analyzed by silica TLC (iPrOH:H_2O:NH_4OH (6:1:3 v/v/v)) and visualized by *p*-anisaldehyde. The fractions containing the product were concentrated under reduced pressure, and then lyophilized to give 17 mg (20 %) of product **23** as white solid. R_f 0.32 (iPrOH:H_2O:NH_4OH (6:1:3

v/v/v)); **^{1}H NMR** (D_2O, 300 MHz) δ 1.63 (3H, s, CH_3), 3.40 (2H, s, CH_2), 4.17 (2H, d, J = 7 Hz, CH_2), 5.53 (1H, m, CH), 7.22 (1H, m, CH), 7.75 (2H, m, 2CH), 8.32 (1H, d, J = 5Hz, CH); **^{13}C NMR** (D_2O, 75 MHz) δ 14.73, 47.75, 62.32, 62.39, 122.03, 122.15, 126.26, 126.37, 134.91, 138.55, 149.02, 158.91; **^{31}P NMR** (D_2O, 125 MHz) δ -9.73 (d, J = 22 Hz), -6.92 (d, J = 22 Hz); **HRMS** (MALDI) calcd. for $C_{10}H_{14}NO_7S_2P_2$ [M-H] 385.9692, found 385.9702.

(*E*)-(4-Mercapto-3-methyl-but-2-enyl) diphosphate ammonium salt (24). To diphosphate **23** (50 mg, 0.12 mmol) in 100 mM NH_4HCO_3 (1 mL) was added DTT (55 mg, 0.36 mmol) in 100 mM NH_4HCO_3 (1 mL). The mixture was allowed to stir for 1 h at room temperature. The mixture was lyophilized, and the residue was chromatographed on cellulose with elution by 100 mM NH_4HCO_3:iPrOH (2:8 v/v). The fractions were analyzed by silica TLC (iPrOH:H_2O:NH_4OH (6:1:3 v/v/v)) and visualized by *p*-anisaldehyde. The fractions containing the product were concentrated under reduced pressure, and then lyophilized to give 17 mg (90%) of product **24** as white solid. R_f 0.32 (iPrOH:H_2O:NH_4OH (6:1:3 v/v/v)); **^{1}H NMR** (D_2O, 300 MHz) δ 179 (3H, s, CH_3), 3.18 (2H, s, CH_2), 4.47 (2H, t, J = 8 Hz, CH_2), 5.62 (1H, m, CH); **^{13}C NMR** (D_2O, 75 MHz) δ 14.45, 32.37, 62.61, 62.68, 121.89, 122.00, 125.12; **^{31}P NMR** (D_2O, 125 MHz) δ -9.73 (d, J = 22 Hz), -6.92 (d, J = 22 Hz); **HRMS** (MALDI) calcd. for $C_5H_{11}O_7SP_2$ [M-H] 276.97062, found 276.97112.

References

1. Rohmer, M.; Knani, M.; Simonin, P.; Sutter, B.; Sahm, H. *Biochem. J.,* **1993**, *295*, 517-524.

2. (a) Hecht, S.; Eisenreich, W.; Adam, P.; Amslinger, S.; Kis, K.; Bacher, A.; Arigoni, D.; Rohdich, F. *Proc. Natl. Acad. Sci. USA*, **2001**, *98*, 14837-14842. (b) Adam, P.; Hecht,

S.; Eisenreich, W.; Kaiser, J.; Gräwert, T.; Arigoni, D.; Bacher, A.; Rohdich, F. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 12108-12113.

3. (a) Seemann, M.; Tse Sum Bui, B.; Wolff, M.; Tritsch, D.; Campos, N.; Boronat, A.; Marquet, A.; Rohmer, M. *Angew. Chem., Int. Ed*., **2002**, *41*, 4337-4339. (b) Wolff, M.; Seemann, M.; Tse Sum Bui, B.; Frapart, Y.; Tritsch, D.; Garcia-Estrabot, A.; Rodriguez-Concepción, M.; Boronat, A. Marquet, A.; Rohmer, M. *FEBS Lett*., **2003**, *541*, 115-120.

4. Seemann, M.; Wegner, P.; Schünemann, V.; Tse Sum Bui, B.; Wolff, M.; Marquet, A.; Trautwein, A. X.; Rohmer, M. *J. Biol. Inorg. Chem*., **2005**, *10*, 131-137.

5. Puan, K. J.; Wang, H.; Dairi, T.; Kuzuyama, T.; Morita, C. T. *FEBS Lett*., **2005**, *579*, 3802-3806.

6. Gräwert, T.; Kaiser, J.; Zepeck, F.; Laupitz, R.; Hecht, S.; Amslinger, S.; Schramek, N.; Schleicher, E.; Weber, S.; Haslbeck, M.; Buchner, J.; Rieder, C.; Arigoni, D.; Bacher, A.; Eisenreich, W.; Rohdich, F. *J. Am. Chem. Soc*., **2004**, 126, 12847-12855.

7. Wolff, M.; Seemann, M.; Grosdemange-Billiard, C.; Tritsch, D.; Campos, N.; Rodriguez-Concepcion, M.; Boronat, A.; Rohmer, M. *Tetrahedron Lett*., **2002**, *43*, 2555-2559.

8. Seemann, M.; Campos, N.; Rodriguez-Concepción, M.; Ibañez, E.; Duvold, T.; Tritsch, D.; Boronat, A.; Rohmer, M. *Tetrahedron Lett*., **2002**, *43*, 1413-1415.

9. Fox, D. T.; Poulter, C. D. *J. Org. Chem.,* **2002**, *67*, 5009-5010.

10**.** Kollas, A.-K.; Duin, E. C.; Eberl, M.; Altincicek, B.; Hintz, M.; Reichenberg, A.; Henschker, D.; Henne, A.; Steinbrecher, I.; Ostrovsky, D. N.; Hedderich, R.; Beck, E.; Jomaa, H.; Wiesner, J. *FEBS Lett.,* **2002**, *532*, 432-436.

11. Giner, J.-L.; Jaun, B.; Arigoni, D. *Chem. Commun*., **1998**, *17*, 1857–1858.

12. Charon, L.; Hoeffler, J. F. ; Pale-Grosdemange, C.; Lois, L. M.; Campos, N. ; Boronat, A.; Rohmer, M. *Biochem. J*., **2000**, *346*, 737–742.

13. Rohdich, F.; Hecht, S.; Bacher, A.; Eisenreich, W. *Pure Appl. Chem*., **2003**, *75*, 393–405.

14. Hintz, M.; Reichenberg, A.; Altincicek, B.; Bahr, U.; Gschwind, R. M.; Kollas, A. K.; Beck, E.; Wiesner, J.; Eberl, M.; Jomaa, H. *FEBS Lett*., **2001**, *509*, 317-322.

15. Rohdich, F.; Hecht, S.; Gärtner, K.; Adam, P.; Krieger, C.; Amslinger, S.; Arigoni, D.; Bacher, A.; Eisenreich W. *Proc. Natl. Acad. Sci. USA,* **2002**, *99*, 1158–1163.

16. Altincicek, B.; Duin, E. C.; Reichenberg, A.; Hedderich, R.; Kollas, A.-K.; Hintz, M.; Wagner, S.; Wiesner, J.; Beck, E.; Jomaa, H. *FEBS Lett.*, **2002**, 532, 437-440.

17. Rohdich, F.; Zepeck, F.; Adam, P.; Hecht, S.; Kaiser, J.; Laupitz, R.; Gräwert, T.; Amslinger, S.; Eisenreich, W.; Bacher, A.; Arigoni, D. *Proc. Natl. Acad. Sci. USA,* **2003**, *100*, 1586–1591.

18. Laupitz, R.; Gräwert, T.; Rieder, C.; Zepeck, F.; Bacher, A.; Arigoni, D.; Rohdich, F.; Eisenreich, W. *Chem. Biodiv*., **2004**, *1*, 1367-1376.

19. (a) Dunlap, N. K.; Mergo, W.; Jones, J. M.; Carrick, J. D. *Tetrahedron Lett.*, **2002**, *43*, 3923-3925. (b) Outram, H. S.; Raw, S. A.; Taylor, J. K. *Tetrahedron Lett.*, **2002**, *43*, 6185-6187.

20. Gupta, D. N.; Hodge, P.; Davies, J. E. *J. Chem. Soc Perkin Trans I.*, **1981**, 2970-2973.

21. (a) Fontana, A. *J. Org. Chem.*, **2001**, *66*, 2506-2508. (b) Fontana, A.; Messina, R.; Spinella, A.; Cimino, G. *Tetrahedron Lett*., **2000**, *41*, 7559-7562.

22. Mitsunobu, O. *Synthesis*, **1981**, 1-28.

23. Osby, J. O.; Martin, M. G.; Ganem, B. *Tetrahedron Lett*., **1984**, 25, 2093-2096.

24. Turek, T. C.; Gaon, I.; Strickland, C. L.; Distefano, M. D. *J. Org. Chem.*, **2001**, *66*, 3253-3264.

25. Green, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, Wiley-Interscience, New York, **1999**, 506-5207, 736-739.

26. Davisson, V. J.; Woodside, A. B.; Neal, T. R.; Stremler, K. E.; Muehlbacher, M.; Poulter, C.D. *J. Org. Chem*., **1986**, *51*, 4768-4779.

27. Cramer, F.; Böhm, W. *Angew. Chem.*, **1959**, 71, 775-780.

28. Danilov, L. L.; Druzhinina, N. A.; Kalinchuk, S. D.; Maltsev, S. D.; Shibaev, V. N. *Chem. Phys. Lipids,* **1989**, *51*, 191-203.

29. Keller, R. K.; Thompson, R. *J. of Chromatography*, **1993**, *645*, 161-167.

30. Brånalt, J.; Kvarnstrom, I.; Classon, B.; Samuelsson, B. *J. Org. Chem.*, **1996**, *61*, 3604-3610.

31. Corey, E. J.; Kim, C. V.; Taheeda, M. *Tetrahedron Lett.*, **1972**, 4339-4341.

32. Jones, L. R.; Goun, E. A.; Shinde, R.; Rothbard, J. B.; Contag, C. H.; Wender, P. A. *J. Am. Chem. Soc.,* **2006**, *128*, 6526 -6527.

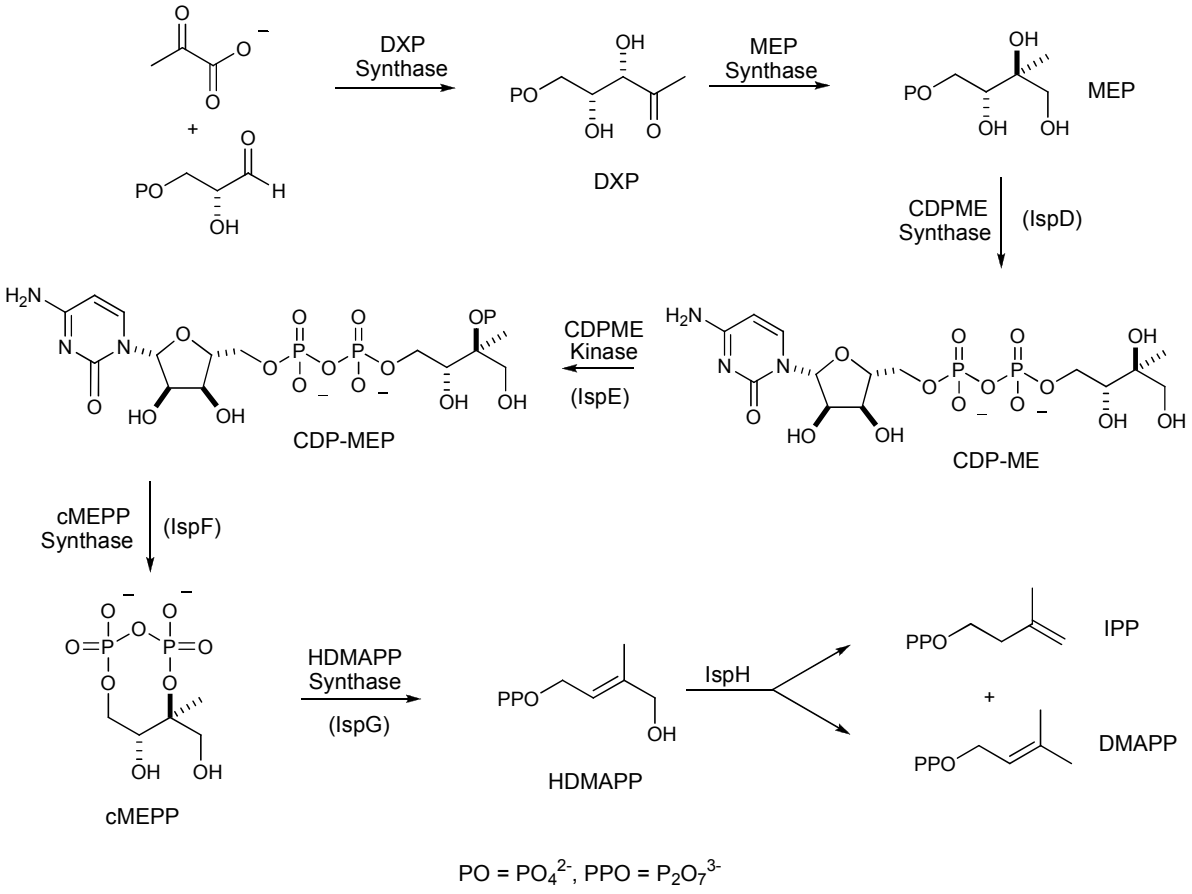
CHAPTER 3

SYNTHESIS AND EVALUATION OF METHYLERYTHRITOL PHOSPHATE ANALOGUES

Background

The MEP pathway begins with the condensation of glyceraldehyde phosphate and pyruvate to form 1-deoxy-D-xylulose-5-phosphate (DXP) catalyzed by DXP synthase. DXP is then rearranged and reduced by MEP synthase to produce methylerythritol phosphate (MEP). MEP is converted into methylerythritol cyclodiphosphate (cMEPP) by the consecutive actions of three enzymes: CDP-ME synthase, CDP-ME kinase, cMEPP synthase. A reductive ring opening of cMEPP provides hydroxydimethylallyl diphosphate (HDMAPP). The IspH catalyzes the final step of the MEP pathway – formation of IPP and DMAPP. It is interesting to note that in contrast to the mevalonate (MVA) pathway, organisms utilizing the MEP pathway do not require IPP isomerase activity. However, the IPP isomerase is found in most organisms (**Scheme 3.1**).[1]

This chapter describes the synthesis and evaluation of analogues as alternative substrates for methylerythritol phosphate (MEP): aminomethylerythritol phosphate (NMEP), methylerythritol thiophosphate (MESP), and thiomethylerythritol phosphate (SMEP) (**Figure 3.1**).



Scheme 3.1. Methylerythritol Phosphate (MEP) Pathway.

Several inhibitors, which are diphosphate analogues, have been synthesized by replacing the diphosphate group with less reactive or unreactive moieties, such as phosphonophosphates,[2] phosphonophosphinates,[3] and bisphosphonates.[4] Typically, these compounds are not especially potent prenyltransferase inhibitors, since the pKa of these groups are different from the normal diphosphates. In contrast, thiophosphate analogues of isopentenyl diphosphate (IPP), dimethylallyl diphosphate (DMAPP), geranyl diphosphate (GPP), farnesyl diphosphate (FPP), and geranylgeranyl diphosphate (GGPP) have pKa's that are similar to the normal diphosphates.[5] The thiophosphate is poorer

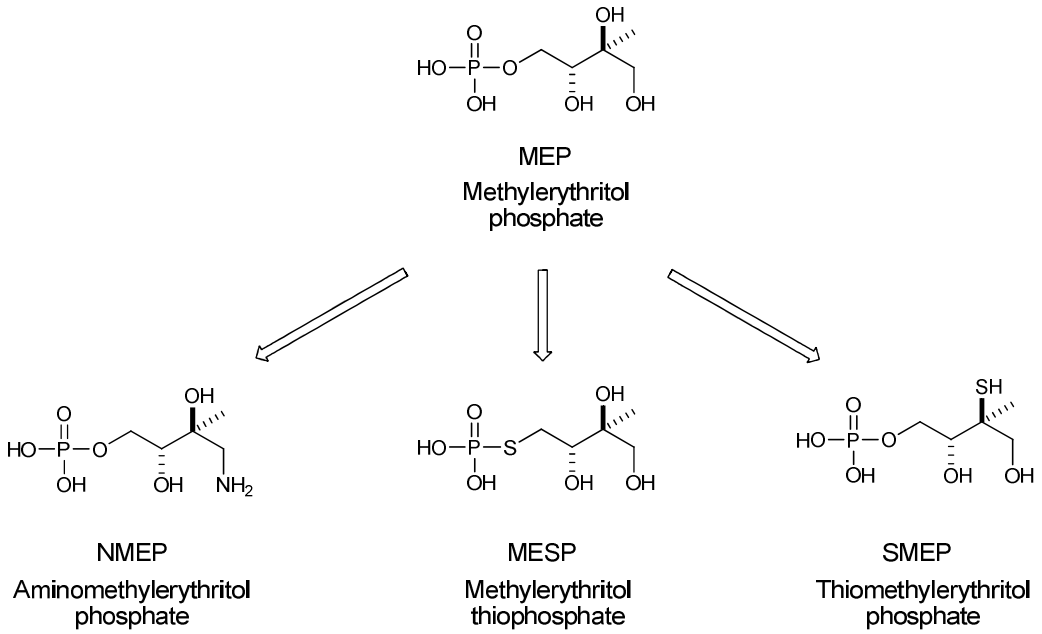


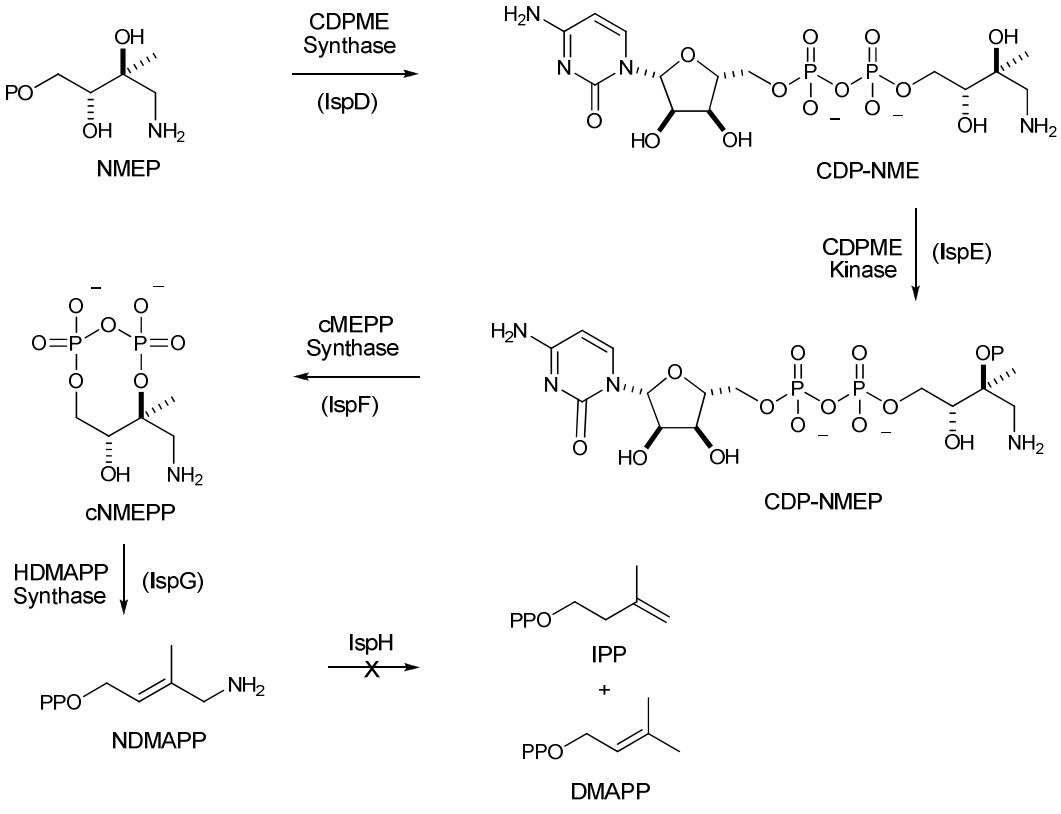
Figure 3.1. Proposed analogues for MEP.

leaving group. Kinetic studies revealed that geranyl thiodiphosphate (GSPP) binds to the allylic site in FPPase with excellent selectivity and affinity, and is an efficient competitive inhibitor of FPPase (the reaction of IPP with GSPP is estimated to be 10^5 times slower than for GPP).

In this chapter, the question of how changes in MEP affect the enzymatic conversion of the alternative substrates to cMEPP analogues by the consecutive action of three proteins in the MEP pathway, IspD, IspE, and IspF is addressed. The analogues were evaluated as alternative substrates or inhibitors of IspDF (bifunctional protein, with IspD and IspF catalytic subunits) and IspE from *Agrobacterium tumefaciens*.

Rationale for Aminomethylerythritol Phosphate

The primary hydroxy group of MEP is not directly involved in the enzymatic conversion of MEP to HDMAPP except perhaps through binding. Aminomethylerythritol phosphate analogue (NMEP) could potentially be recognized by the respective enzymes and converted into cNMEPP **(Scheme 3.2)**. The mechanism for the conversion of cMEPP to HDMAPP (**Scheme 3.1**) catalyzed by IspG is not known. Two suggestions for this step were discussed in the previous chapter (Chapter 2, **Schemes 2.2** and **2.3**).[6] Accordingly, the reaction may proceeds by either a cationic or a radical mechanism. In both cases, the primary hydroxyl group of MEP is oxidized to an aldehyde.

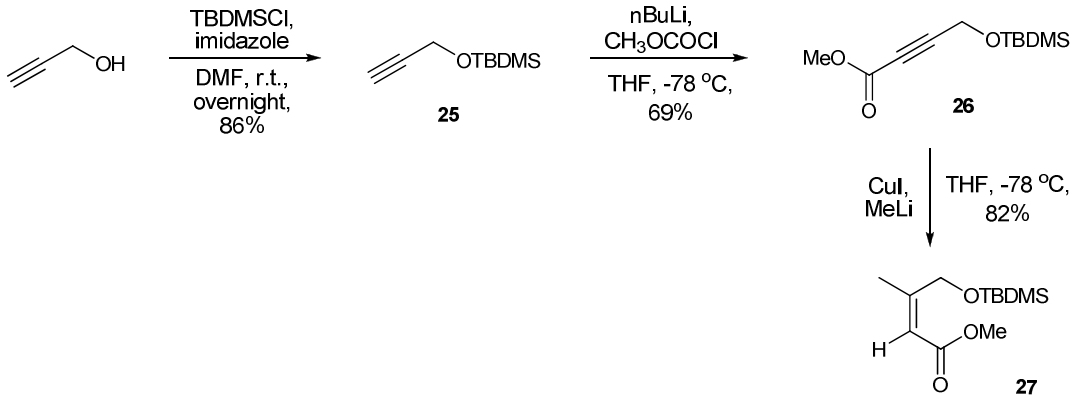


Scheme 3.2. Proposed metabolism for NMEP.

In a similar manner, the amino group in NMEP could be oxidized to an imine. However, the amine should be protonated at physiological pH and the ammonium species should resist oxidation. Therefore, NMEP might be converted to intermediates that inhibit the enzymes starting from IspG in the pathway. Additionally, this analogue will ultimately inhibit the IspH when it is converted to NDMAPP. The synthesis and activity of the aminodimethylallyl diphosphate (NDMAPP) with IspH is discussed separately in Chapter 2. It was previously shown by our collaborators in France that NDMAPP is a potent nanomolar inhibitor of IspH.

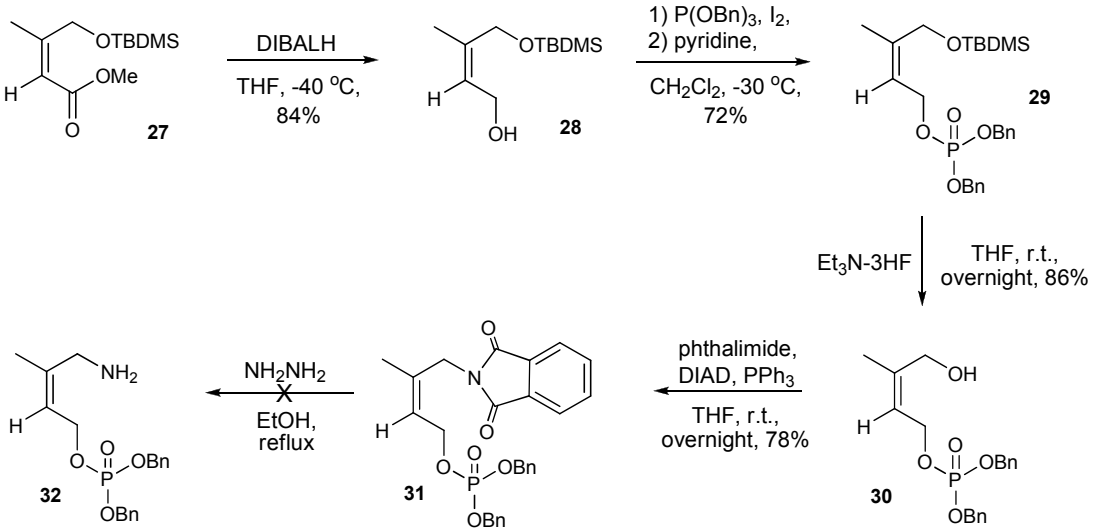
Synthesis of Aminomethylerythritol Phosphate

The synthesis of NMEP begins with the synthesis of *Z* α,β-unsaturated ester **27** from propargyl alcohol, as described in the literature (**Scheme 3.3**).[7] Propargyl alcohol was protected as a TBDMS ether. The acetylenic proton of **25** was removed with *n*BuLi and the resulting anion was treated with chloroformate to provide ester **26**. The ester **27** was prepared from **26** by the method of Corey and Katzenellenbogen,[8] to give the *Z* isomer exclusively. It should be noted that exclusive formation of the Z isomer requires slow addition of a 1M solution of ester **26** in THF to the methyl cuprate at -78 ^{o}C. The yield of the reaction is increased when the reaction is quenched with MeOH that has been cooled to -78 ^{o}C.[8]



Scheme 3.3. Synthesis of *Z* α,β-unsaturated ester **27**.

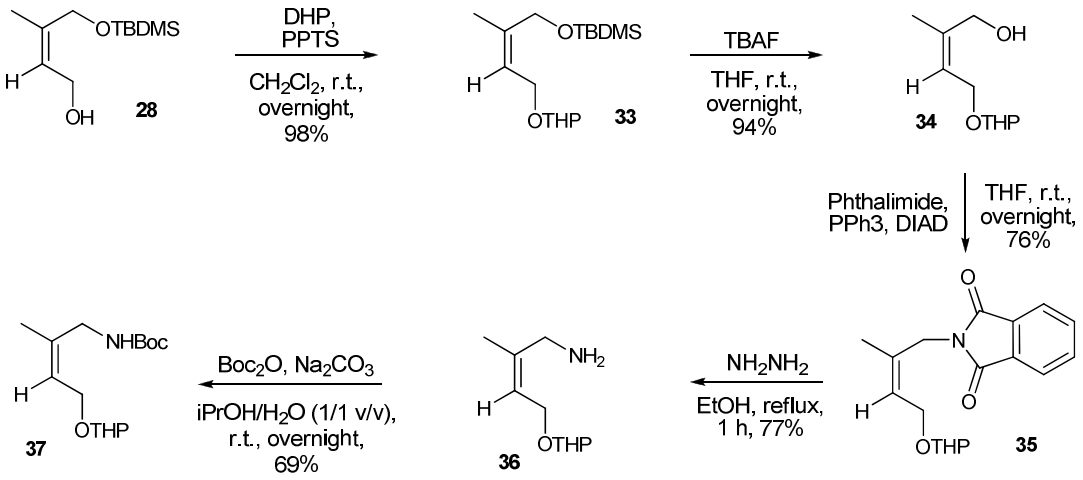
Reduction of **27** ester with DIBALH at -40 °C gave alcohol **28**, which was then protected with the THP protecting group (**Scheme 3.4**). Alcohol **29** was phosphorylated using benzyl phosphite-iodine procedure,[9] and the TBDMS group was removed with the triethylamine hydrofluoride complex[10] to give **30**. The amino group was introduced by treating alcohol **30** with phthalimide under Mitsunobu conditions.[11] However, all attempts to remove the phthalimide group from **31** led to decomposition.



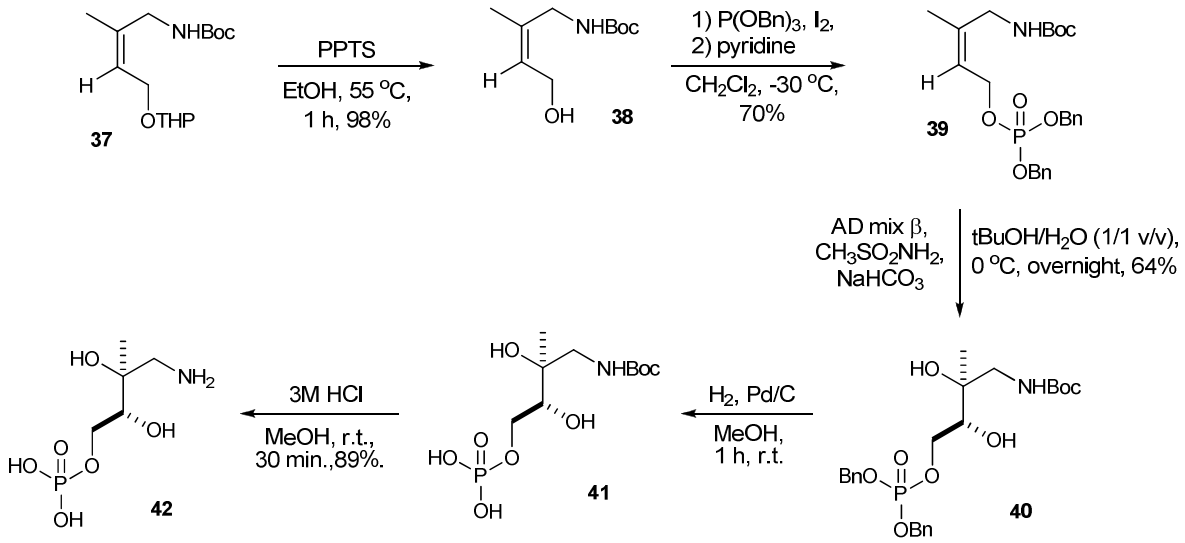
Scheme 3.4. Synthesis of *Z* amino phosphate ester **32**.

Alcohol **28** was then protected with THP, and the TBDMS group of **33** was removed with the triethylamine hydrofluoride complex (**Scheme 3.5**). The amino group was then introduced by treating the alcohol **34** with phthalimide under Mitsunobu conditions.[11] The phthalimide moiety was removed with hydrazine and the resulting amine was protected with di-*tert*-butyl dicarbonate to give **37**.

After removal of the THP group, alcohol **38** was phosphorylated using benzyl phosphite-iodine (**Scheme 3.6**).[9] The Sharpless asymmetric dihydroxylation[12] established two chiral centers of **40**, according to the Sharpless mnemonic device.[13] The *er* ratio was found to be 12/1 using chiral HPLC. NMEP was obtained after deprotection of **40** by treatment with H_2 and Pd/C to remove the benzyl groups, followed by HCl to remove the Boc group (**Scheme 3.6**).



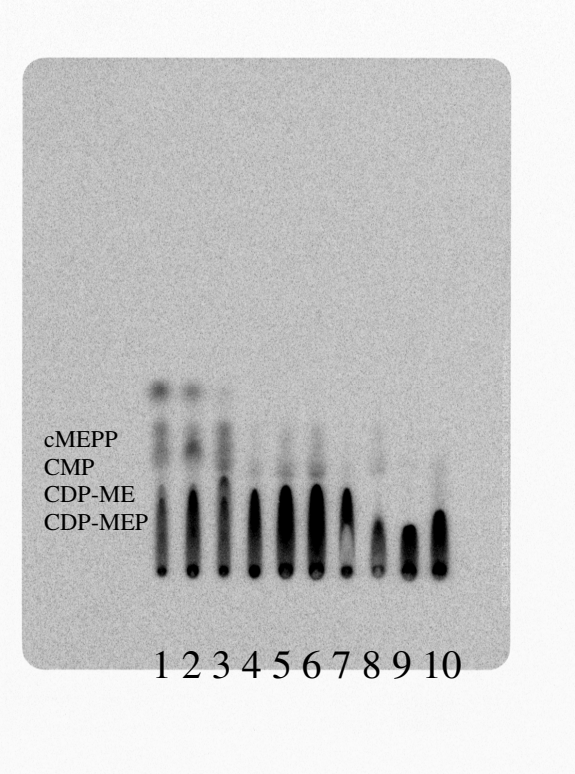
Scheme 3.5. Synthesis of Boc protected amine **37**.



Scheme 3.6. Synthesis of NMEP.

Enzymatic Evaluation of Aminomethylerythritol Phosphate

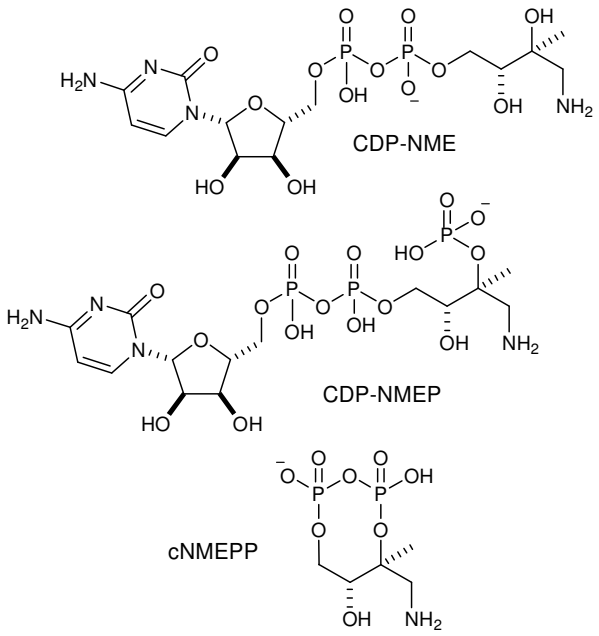
NMEP was tested as an alternative substrate and as an inhibitor of IspDF and IspE for the sequence of reactions NMEP → CDP-NME → CDP-NMEP → cNMEPP. These transformations were detected by the ^{32}P assay in presence of radiolabelled and cold CTP and ATP and by LCMS. The experiments with ^{32}P were analyzed by TLC as described in the literature.[14,15] A typical TLC analysis is shown in **Figure 3.2**. Samples were preincubated for 5 min in the presence of all substrates, except for radiolabeled ATP and CTP. Reactions were initiated by addition of CTP. After 15 min, the reactions were quenched with 50 µL of methanol and were put on ice. TLC (Polygram Sil N-HR; Macherey & Nagel) was performed by spotting 3.5 µL of the reaction mixture and developing the plates with 1-propanol/ethyl acetate/H_2O (6:1:3 v/v/v).



Reaction conditions: lane 1: MEP + CTP, ATP, IspDF, IspE; lane 2: MEP + CTP, ATP, IspDF, IspE; lane 3: MEP + CTP, ATP, mutant IspD, IspE; lane 4: NMEP + CTP, ATP, IspDF, IspE; lane 5: NMEP + CTP, ATP, mutant IspD; lane 6: NMEP + CTP, ATP, mutant IspD, IspE; lane 7: NMEP + CTP cold, ATP, IspDF, IspE; lane 8: NMEP + CTP, ATP cold, IspDF, IspE; lane 9: CTP; lane 10: ATP.

Figure 3.2. TLC plate showing the different products following incubation of MEP and NMEP (500 μM), IspDF (4.8 μM), IspE (6.2 μM), γ-[^{32}P]ATP (150 μM, 320 μCi/μmol), α-[^{32}P]CTP (150 μM, 40 μCi/μmol) after 1 h reaction.

As seen from **Figure 3.2**, the TLC data indicate that NMEP is a substrate for the IspDF and IspE. Potential intermediates are listed in **Figure 3.3**. To establish the identity of the products, a set of LCMS experiments were performed as shown in **Figures 3.4** and **3.5** following procedures described in the literature.[16] After 30 min, reaction was quenched with MeOH and the proteins were removed by filtration. A 15 μL sample was injected into the HPLC column and the eluents were analyzed by mass spray analysis. CDP-NME, CDP-NMEP, cNMEPP were selected by extracting corresponding masses from the spectrum. The comparison of isotope distribution of the extracted compounds led us to assign each metabolite. These LCMS experiments establish that NMEP is a



Product of IspD protein - CDP-NME
Chemical Formula: $C_{14}H_{25}N_4O_{13}P_2^-$
Exact Mass: 519.09
Molecular Weight: 519.32
m/z: 519.09 (100.0%), 520.09 (17.1%), 521.09 (2.9%), 521.10 (1.2%)

Product of IspE protein - CDP-NMEP
Chemical Formula: $C_{14}H_{26}N_4O_{16}P_3^-$
Exact Mass: 599.06
Molecular Weight: 599.30
m/z: 599.06 (100.0%), 600.06 (16.1%), 601.06 (4.7%), 600.05 (1.5%)

Product of IspF protein - cNMEPP
Chemical Formula: $C_5H_{12}NO_8P_2^-$
Exact Mass: 276.00
Molecular Weight: 276.10
m/z: 276.00 (100.0%), 277.01 (5.9%), 278.01 (1.8%)

Figure 3.3. Potential intermediates for enzymatic conversion of NMEP to cNMEPP.

substrate for both reactions catalyzed by IspDF, and for IspE to give cNMEPP using selective ion-monitoring.

Rationale for Methylerythritol Thiophosphate

The considerations outlined in the rationale section for NMEP analogue led us to consider a methylerythritol thiophosphate analogue, where the phosphate oxygen atom attached to the alkyl group is replaced with sulfur. Since it is known that thiophosphate analogues of isopentenyl diphosphate (ISPP) inhibit prenyl transfer,[5] MESP might serve as a surrogate for DMASPP by functioning as an alternative substrate for the MEP pathway enzymes (**Scheme 3.7**). Isoprenoid biosynthesis would then be blocked during chain elongation.

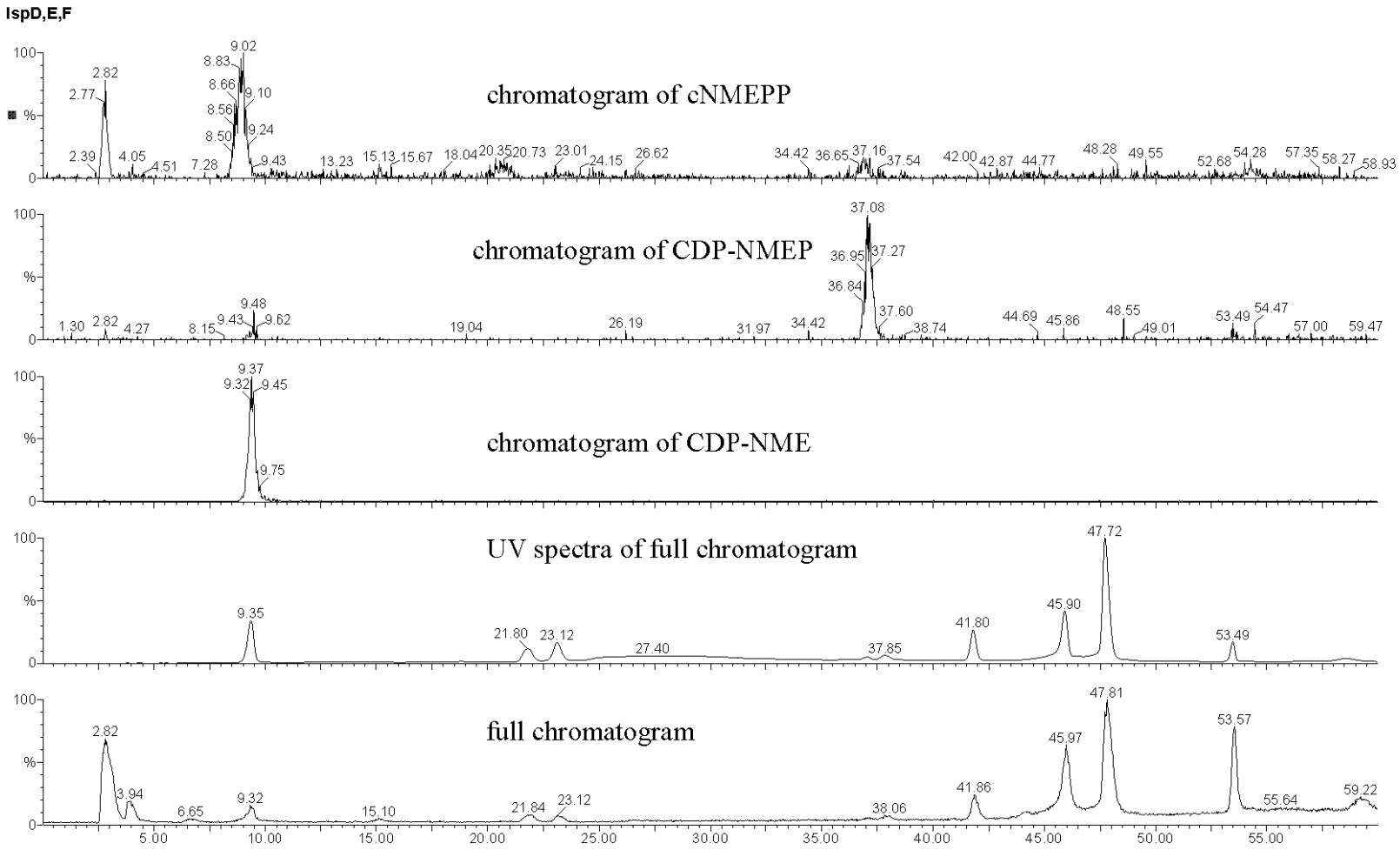


Figure 3.4. LC-MS chromatograms of the different products formed after incubation of NMEP with IspDF, mutant IspD, IspE: consecutively cNMEPP, CDP-NMEP, CDP-NME, UV spectra, and full chromatogram.

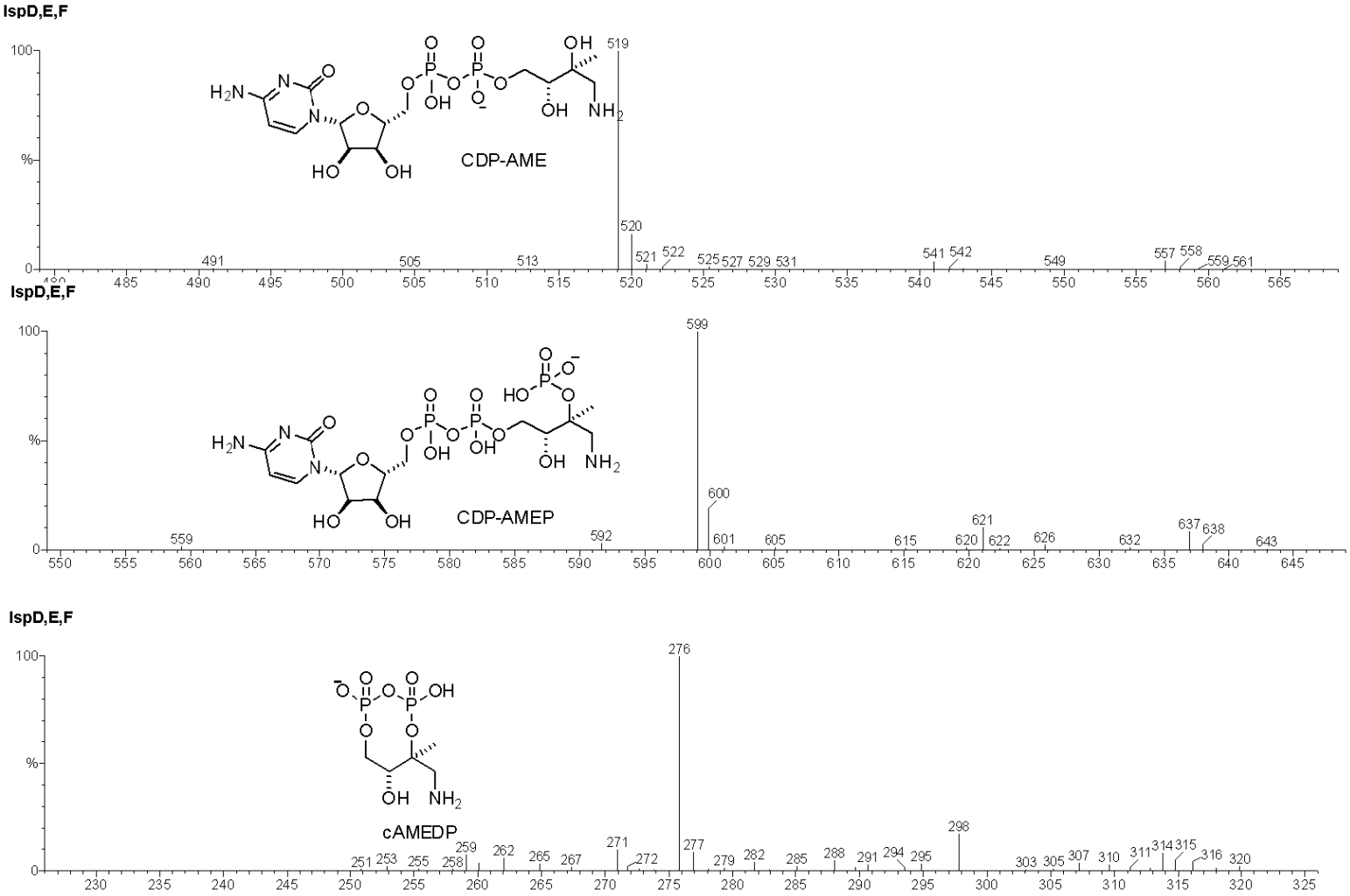
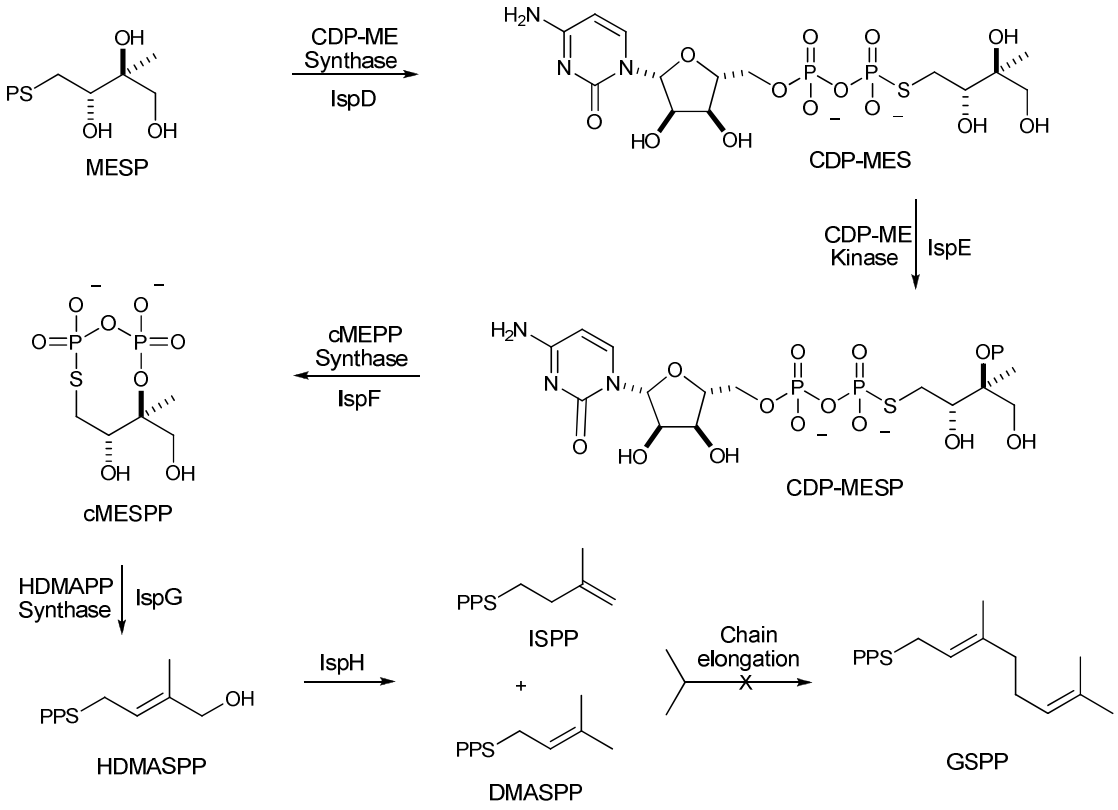


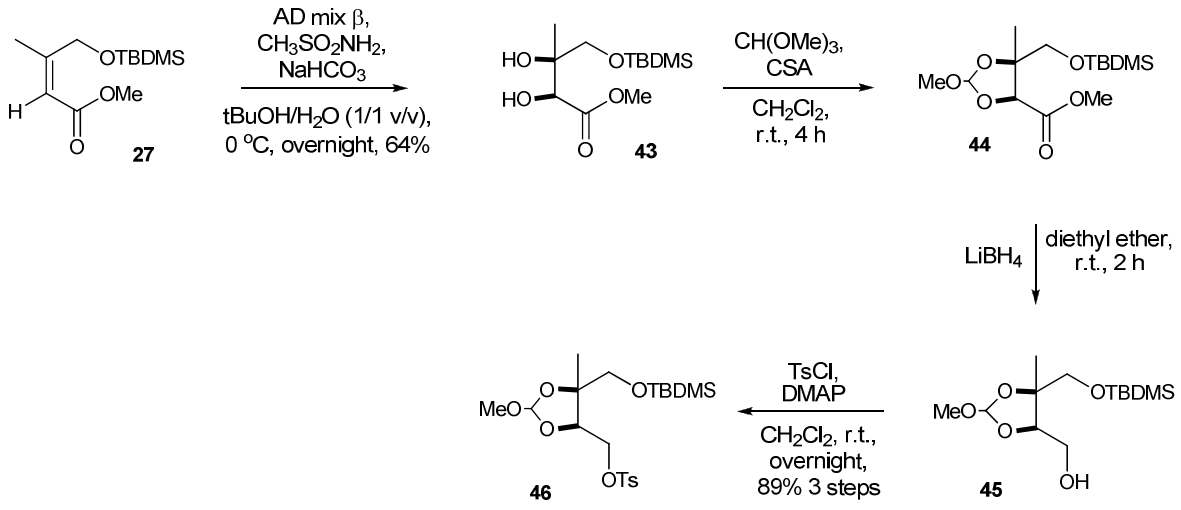
Figure 3.5. The ions of the intermediates for NMEP conversion to cNMEPP.



Scheme 3.7. Proposed metabolism for MESP.

Synthesis of Methylerythritol Thiophosphate

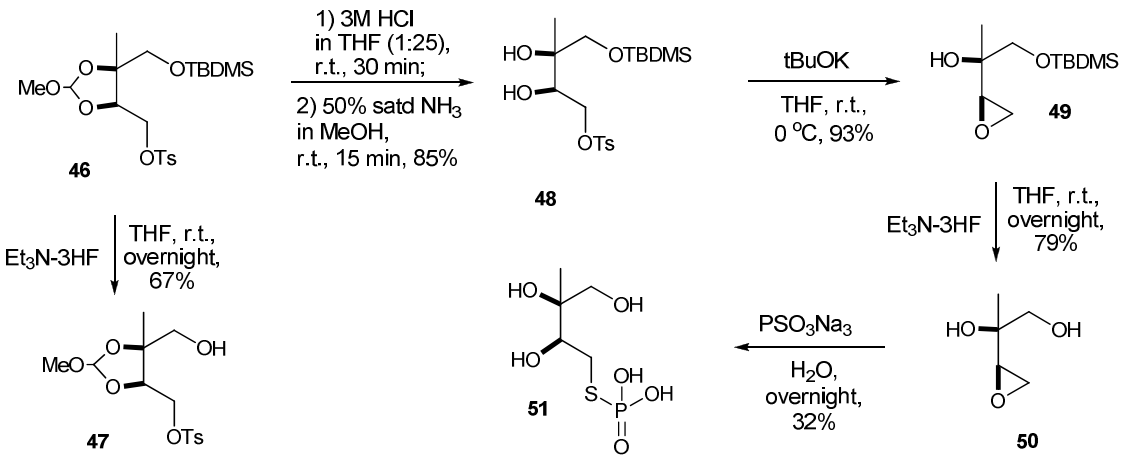
The synthesis of MESP is shown in **Schemes 3.8** and **3.9**. Ester **27** from **Scheme 4** was asymmetrically dihydroxylated using Sharpless reaction (**Scheme 3.8**).[12] The Mosher ester analysis of **43** showed 18/1 ratio between enantiomers.[17] Diol **43** was first protected as an acetonide. However, the protecting group would not be removed without decomposition of the molecule. Therefore, the diol **43** was protected as an orthoester **44**. Although the reaction proceeded in quantative yield, **44** was unstable on a silica column. After the purification, the product was obtained in 51% yield. In order to improve yield, we omitted the purification step. Ester **44** was reduced with $LiBH_4$ to alcohol **45** in



Scheme 3.8. Synthesis of orthoester **46**.

a quantitative yield. Again, the alcohol was unstable on silica, and **45** was obtained in 61% yield after purification. Again, in order to improve yield, we omitted the purification step; only aqueous workup was performed for alcohol **45**. Alcohol **45** was converted into tosylate **46** with isolated yield of 89% yield for the three steps. Tosylate **46** was sufficiently stable to be chromatographed on silica.

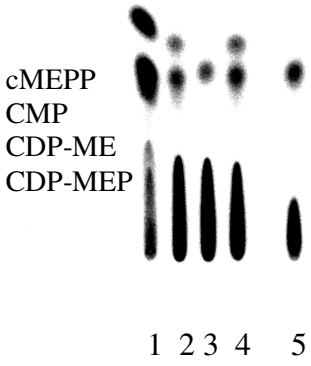
The orthoester was cleaved first by mild treatment with HCl to give a formate ester, followed by hydrolysis with ammonia in CH_3OH to give **48**. It is interesting to note that the TBDMS protecting group was cleaved by triethyl amine hydrofluoride complex in the presence of the orthoester and tosylate group to produce **47**. Using *t*BuOK as a bulky base, diol **48** was converted to epoxide **49**. The TBDMS group was removed to give epoxide **50**, and **50** was opened with trisodium thiophosphate salt to give MESP (**51**) (**Scheme 3.9**). The Mosher ester analysis of **50** showed 17/1 enantiomeric ratio.



Scheme 3.9. Synthesis of MESP.

Enzymatic Evaluation of Methylerythritol Thiophosphate

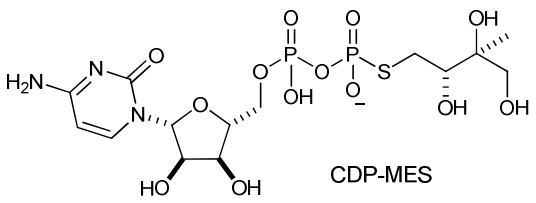
MESP was evaluated as an alternative substrate for IspDF, and IspE using the procedure described for NMEP in transformation: MESP → CDP-MESP → CDP-MESP → cMESPP. The transformation was followed by the ^{32}P TLC in presence of radiolabelled and cold CTP and ATP[14,15] and by LCMS. Samples were preincubated for 5 min in the presence of all substrates, except for radiolabeled ATP and CTP. Reactions were initiated by addition of CTP. After 15 min, the reactions were quenched with 50 µL of methanol and were put on ice. TLC analysis was performed by spotting 3.5 µL of the reaction mixture and developing the plates with 1-propanol/ethyl acetate/H_2O (6:1:3 v/v/v). As it can be seen from **Figure 3.6**, MESP is a better alternative substrate for the IspDF and IspE than NMEP. Possible intermediates are listed in **Figure 3.7**. The structures of the metabolites were confirmed by LCMS, as shown in **Figures 3.8** and **3.9**.[16]



Reaction conditions: lane 1: MEP + CTP, ATP, IspDF, IspE; lane 2: MESP + CTP, ATP, IspDF, IspE; lane 3: MESP + CTP, ATP, mutant IspD; lane 4: MESP + CTP, ATP, mutant IspD, IspE; lane 5: MESP + CTP, ATP cold, IspDF, IspE.

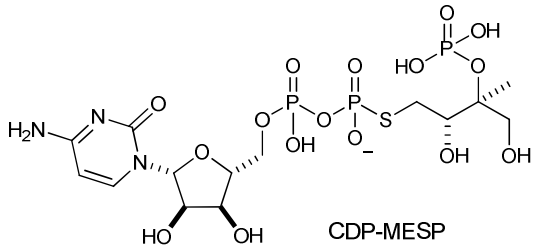
Figure 3.6. TLC plate showing the different products following incubation of MEP and MESP (500 μM), IspDF (4.8 μM), IspE (6.2 μM), γ-[^{32}P]ATP (150 μM, 320 μCi/μmol), α-[^{32}P]CTP (150 μM, 40 μCi/μmol) after 1 h reaction.

After 30 min incubation, the reaction was quenched with MeOH and the proteins were removed by filtration. A 15 μL sample was injected into HPLC and was analyzed by electron mass spray analysis. CDP-MES, CDP-MESP, cMESPP were selected by extracting corresponding masses from the spectrum. The comparison of isotope distribution of the extracted compounds allowed us to assign each metabolite. These LCMS experiments establish that MESP is a substrate for IspDF, IspE and is converted to cyclic MESPP using selective ion-monitoring.



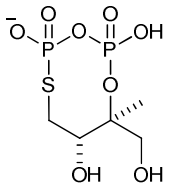
CDP-MES

Product of IspD protein - CDP-MES
Chemical Formula: $C_{14}H_{24}N_3O_{13}P_2S^-$
Exact Mass: 536.05
Molecular Weight: 536.37
m/z: 536.05 (100.0%), 537.05 (17.5%),
538.05 (7.5%), 538.06 (1.2%)



CDP-MESP

Product of IspE protein - CDP-MESP
Chemical Formula: $C_{14}H_{25}N_3O_{16}P_3S^-$
Exact Mass: 616.02
Molecular Weight: 616.35
m/z: 616.02 (100.0%), 617.02 (16.8%), 618.02 (4.8%),
618.01 (4.5%), 619.02 (1.3%), 617.01 (1.1%)



cMESPP

Product of IspF protein - cMESPP
Chemical Formula: $C_5H_{11}O_8P_2S^-$
Exact Mass: 292.97
Molecular Weight: 293.15
m/z: 292.96 (100.0%), 293.97 (5.8%),
294.96 (4.5%), 294.97 (1.8%)

Figure 3.7. Possible intermediates for enzymatic conversion of MESP to cMESDP.

Rationale for Thiomethylerythritol Phosphate

The tertiary hydroxyl group in CDP-ME is phosphorylated by CDP-ME kinase, and is later reductively eliminated when cMEPP is converted to HDMAPP. The substitution of the tertiary hydroxyl group by a thiol moiety should not appreciably affect the kinase activity; however, it may substantially retard elimination step. We were interested in investigating the activity of enzymes in the pathway with the thiomethylerythritol phosphate as the initial substrate. We speculate that our analogue might inhibit HDMAPP synthase (**Scheme 3.10**)

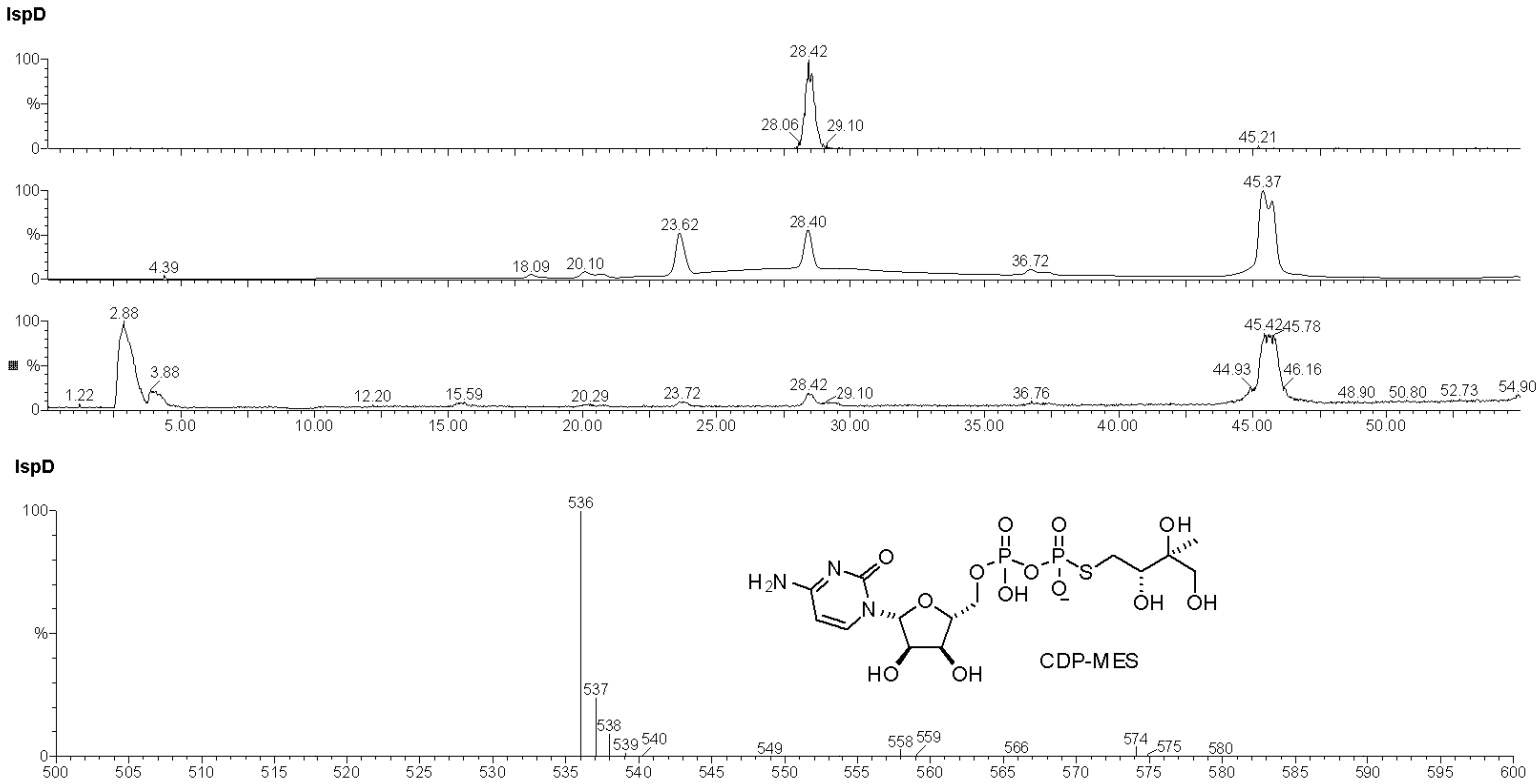


Figure 3.8. Product of IspD catalyzed reaction with MESP analogue.

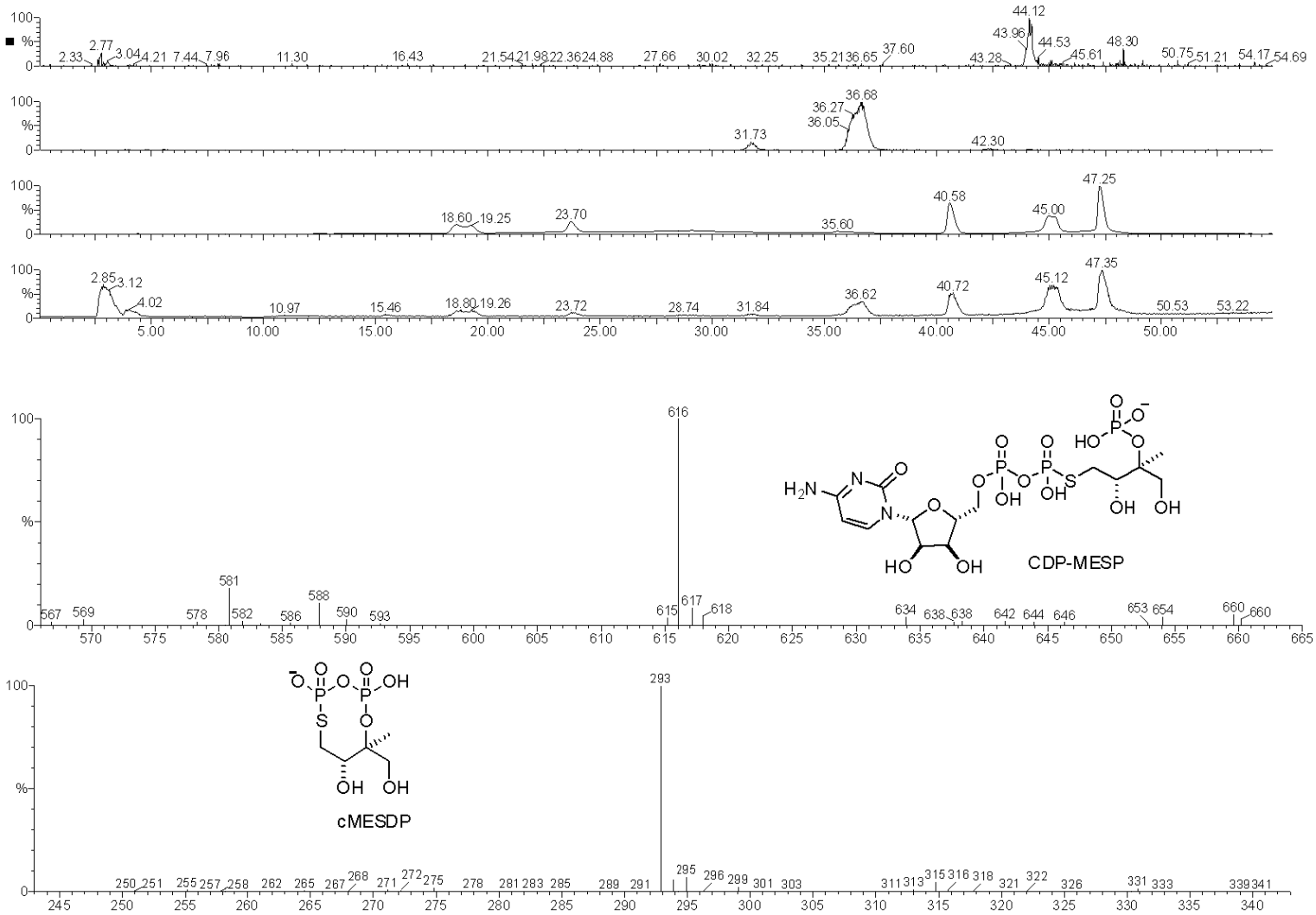
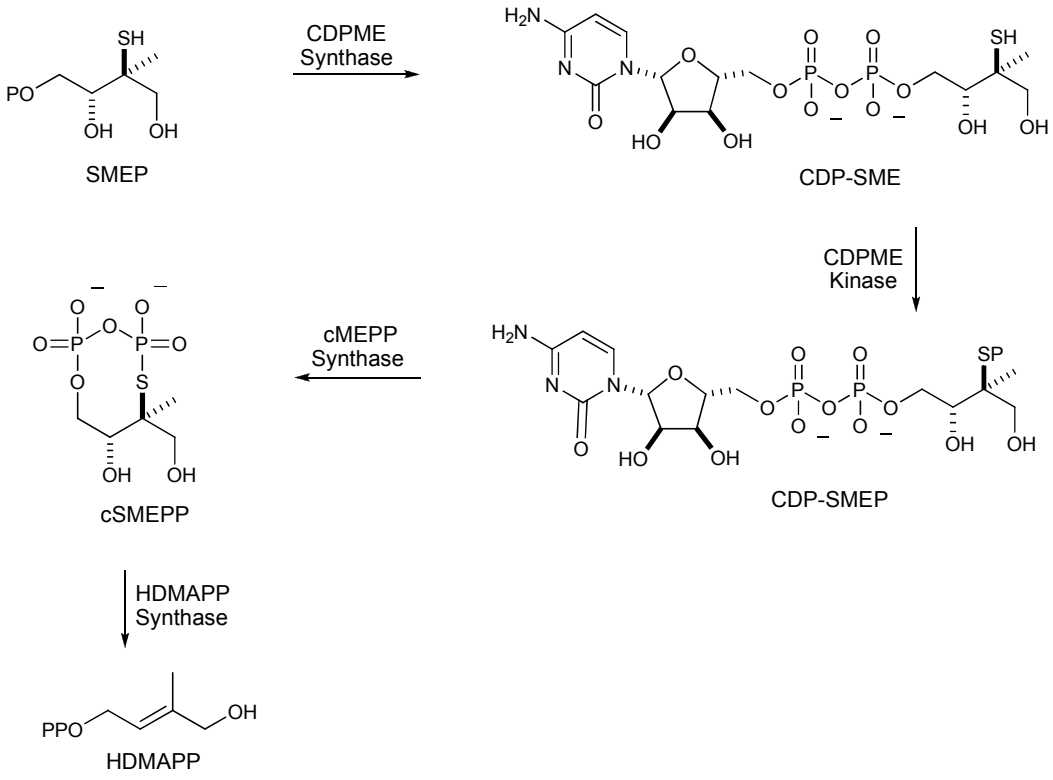


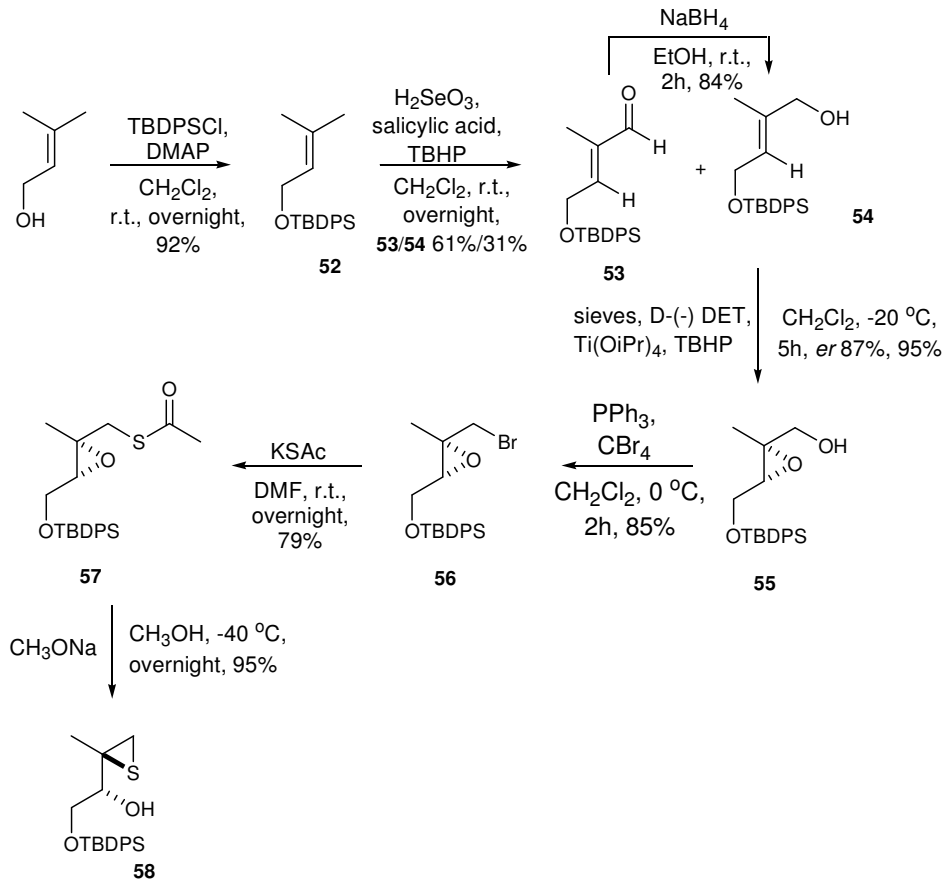
Figure 3.9. Products of IspE and IspF catalyzed reactions.



Scheme 3.10. Proposed metabolism for SMEP.

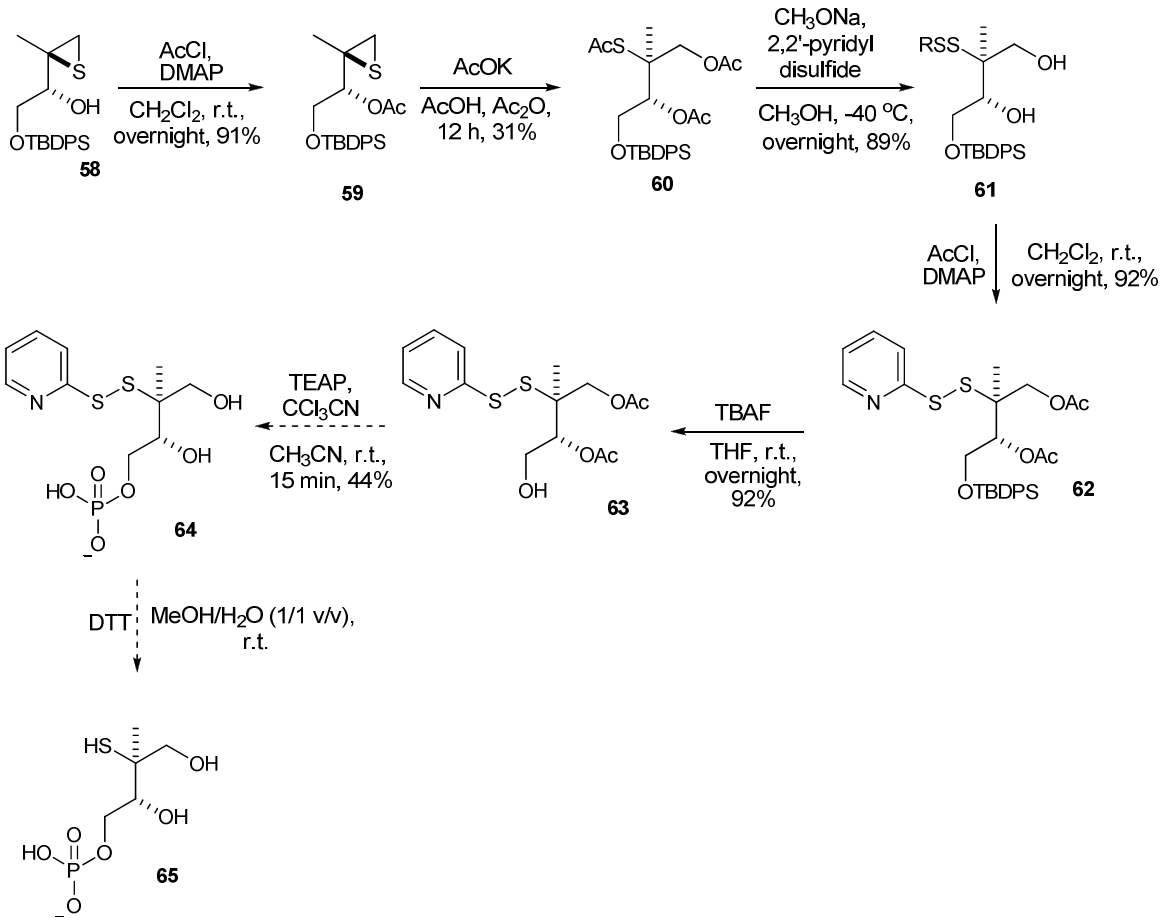
Synthesis of Thiomethylerythritol Phosphate

The synthesis of SMEP is shown in **Schemes 3.11 and 3.12**. 3-Methylbut-2-en-1-ol was treated with TBDPSCl to give **52**, followed by selenious acid oxidation to produce a 1:2 mixture of *E* allylic alcohol **54** and aldehyde **53**. Aldehyde **53** was reduced with $NaBH_4$ to give **54**. Alcohol **54** was epoxidized under Sharpless conditions in 12/1 enantiomeric ratio and resulting epoxy alcohol **55** was converted into bromide **56** using the procedure described by Appel.[18] Bromide **56** was treated with KSAc to produce thioacetate **57**. The acetate moiety was removed with MeONa at -40 ^{o}C, and the resulting epoxy thiol was immediately converted to thiirane **58** by a thio-Payne rearrangement.[19]



Scheme 3.11. Synthesis of thiirane **58**.

The Mosher ester analysis of **58** showed 10/1 enatiomeric ratio (**Scheme 3.11**). The free hydroxyl group of **58** was protected as an acetate to prevent rearrangement during the next step, which involves reflux at high temperature (**Scheme 3.12**). The thiirane ring was opened by refluxing **59** in AcOH/Ac_2O/AcOK to give triacetate **60**.[20] A maximal yield of 30% was obtained after 12 h. Longer times at reflux resulted in accumulation of side products. Consequently, **60** was treated with MeONa at -40 °C in the presence of three equivalents of 2,2'-pyridyl disulfide to trap liberated thiol as a mixed disulfide **61**. The hydroxyl groups of **61** were then acetylated to give **62**. The TBDPS group was



Scheme 3.12. Synthesis of SMEP.

deprotected with TBAF to produce alcohol **63**. The future steps include phosphorylation of **63** and disulfide deprotection to achieve **65**. In summary, the synthesis of NMEP and MESP analogues of MEP was achieved. These analogues were tested as substrates in the MEP pathway and were found to be substrates for CDPME Synthase, CDPME Kinase, and cMEPP Synthase. The synthesis of SMEP was initiated and alcohol **63** was prepared. The future steps include phosphorylation of **63** and disulfide deprotection as demonstrated for the synthesis of NMEP and SDMEPP. These studies will be reported separately.

Experimental Section

General methods

NMR spectra were recorded on a Varian Unity Inova NMR 300 MHz FTNMR interfaced to a Sun Sparcstation 5 computer at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR). All chemical shifts are reported in parts per million (ppm) and coupling constants (*J*) in hertz (Hz). ^{1}H chemical shifts are referenced relative to the TMS peak at δ 0.0 ppm or to the peak of CD_2Cl_2 at 5.32 ppm. The ^{13}C chemical shifts are referenced relative to $CDCl_3$ at δ 77.0 ppm (center peak), the peak of CD_2Cl_2 at 54 ppm (center peak), and for D_2O to MeOH at 49 ppm . ^{31}P chemical shifts are referenced relative to the peak of H_3PO_4 in D_2O at δ 0 ppm.

All commercial reagents were ACS reagent grade and were used without further purification. In general all solvents, reagents, and deuterated solvents were purchased from Fisher Chemicals, Acros, and Aldrich. Methylene chloride and ethanol were distilled from CaH_2 prior to use. All other reagents were of commercial quality from freshly opened containers.

Sharpless asymmetric dihydroxylation (40, 43). In a 25 mL flask was dissolved AD mix β (3.14 g), $NaHCO_3$ (565 mg, 6.73 mmol), and $CH_3SO_2NH_2$ (160 mg, 1.68 mmol) in *t*BuOH:H_2O (1:1 v/v, 12 mL). The solution was cooled to 0 ^{o}C, and an alkene (0.56 mmol) was added. The mixture was stirred overnight at 0 ^{o}C. The reaction was quenched with Na_2SO_3 (3.36 g) at 0 ^{o}C. The temperature was allowed to rise to room temperature, while the solution was stirred for 1 h. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3x20 mL). The combined organic extracts

were dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate.

General preparation and analysis of Mosher esters.[21] The reactions were generally run on a 0.15-mmol scale. A mixture 4-(dimethy1amino)pyridine (18 mg, 0.15 mmol) and triethylamine (100 μL) in CH_2Cl_2 (0.5 mL), was treated with the substrate. Immediately, 30 μL of neat R-(-)-α-methoxy-(trifluoromethy1)phenylacetyl chloride (MTPA chloride) was added. The solution became warm and turned orange. Reactions were concentrated under reduced pressure, and the residue was chromatographed on silica with gradient elution by hexane/ethyl acetate (0% to 20% ethyl acetate). ^{19}F NMR analysis focused on the ratio between fluorine signals.

***tert*-Butyl-dimethyl-prop-2-ynyloxy-silane (25).** TBDMSCl (16.6 g, 0.11 mol) was added slowly to a solution of propargyl alcohol (5.6 g, 0.1 mol) and imidazole (10.2 g, 0.15 mol) in DMF (200 mL) at room temperature. The resulted mixture was stirred overnight at room temperature. The solution was transferred into a separation funnel containing water (1 L) and was extracted with Et_2O (3x200 mL). The combined extracts were dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 2% ethyl acetate) to give 14.6 g (86%) of **25** as colorless oil. R_f 0.64 (hexanes:ethyl acetate 1:1). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 0.13 (6H, s, $2CH_3$), 0.91 (9H, s, $3CH_3$), 2.39 (1H, t, J = 2.3 Hz, CH), 4.31 (2H, d, J = 2.5 Hz, CH_2); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ -5.22, 18.27, 25.77, 51.49, 72.82, 82.39; **HRMS** (FTMS) calcd. for $C_9H_{19}OSi$ $[M+H^+]$ 171.12000, found 171.12018. The NMR data was identical to the data published before.[7]

4-(*tert*-Butyl-dimethyl-silanyloxy)-but-2-ynoic acid methyl ester (26). To a solution of **25** (15 g, 88 mmol) in THF (150 mL) was added 2.5 M solution of *n*BuLi in hexanes (38 mL, 96 mmol) at -78 ^{o}C dropwise via an addition funnel. After the addition was complete, a solution of methyl chloroformate (12.5 g, 132 mmol) in THF (50 mL) was added dropwise over a period of 1 h. The reaction was stirred for 1 h at -78 ^{o}C and then the mixture was warmed to room temperature over 1 h. The mixture was cooled to -20 ^{o}C and quenched with saturated solution of NH_4Cl (200 mL). The layers were separated, and the aqueous layer was extracted with ether (3x50 mL). The combined organic extracts were dried over Na_2SO_4. The solvent was removed at reduced pressure, the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 4% ethyl acetate) to give 18.5 g (92%) of **26** as colorless oil. **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 0.14 (6H, s, $2CH_3$), 0.91 (9H, s, $3CH_3$), 3.78 (3H, s, CH_3), 4.34 (2H, s, CH_2); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ -5.28, 18.20, 25.68, 51.34, 52.72, 76.31, 86.14, 153.75; **HRMS** (FTMS) calcd. for $C_{11}H_{21}O_3Si$ [M+H$^+$] 229.12545, found 229.12517. The NMR data was identical to the data published before.[7]

(*Z*)-4-(*tert*-Butyl-dimethyl-silanyloxy)-3-methyl-but-2-enoic acid methyl ester (27). To a suspension of CuI (8.34 g, 43.8 mmol) in THF (160 mL) at -78 ^{o}C was added 1.5 M solution of MeLi in THF (58.4 mL, 87.58 mmol). The solution became clear and was left to stir for 30 min. A solution of **26** (10 g, 43.8 mmol) in THF (45 mL) was added dropwise to the mixture at -78 ^{o}C. Precise control of temperature was important in order to obtain only *Z* isomer. After the addition was complete, the mixture was stirred for 2 h at the same temperature. The mixture was quenched with MeOH (60 mL) cooled to -78 ^{o}C. Brine (60 mL) was added, and the layers were separated. The aqueous layer was

extracted with ether (3x50 mL). The combined organic extracts were dried over Na_2SO_4. The solvent was removed at reduced pressure, the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 5% ethyl acetate) to give 8.8 g (82%) of **27** as colorless oil. **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 0.08 (6H, s, $2CH_3$), 0.91 (9H, s, $3CH_3$), 1.97-1.98 (3H, m, CH_3), 3.67 (3H, s, CH_3), 4.79-4.8 (2H, m, CH_2), 5.67-5.69 (1H, m, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ -5.49, 18.24, 21.42, 25.84, 50.92, 62.70, 114.39, 161.35, 166.42; **HRMS** (FTMS) calcd. for $C_{12}H_{25}O_3Si$ [$M+H^+$] 245.15675, found 245.15722. The NMR data was identical to the data published before.[7]

(*Z*)-4-(*tert*-Butyl-dimethyl-silanyloxy)-3-methyl-but-2-en-1-ol (28). A solution of DIBALH (1M in hexanes, 4 mL, 4 mmol) in THF (10 mL) was added dropwise via a syringe to a solution of ester **27** (244 mg, 1 mmol) in THF (6 mL) at –78 ^{0}C. The mixture was stirred for 3 h while the temperature was allowed to rise to –40 ^{0}C. After the addition of 0.2 M Na_2CO_3 (20 mL), the mixture was stirred for 30 min at room temperature. The solution was filtrated through celite®, and the filter cake was washed with two 20 mL portions of ether. NaCl was added until separation of phases occurred. The aqueous layer was extracted with ether (3x20 mL). The combined organic extracts were, and dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 30% ethyl acetate) to give 181 mg (84%) of **28** as colorless oil. R_f 0.23 (hexanes:ethyl acetate 4:1); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 0.09 (6H, s, $2CH_3$), 0.91 (9H, s, $3CH_3$), 1.77 (2H, d, J = 1 Hz, CH_2), 1.81 (1H, s broad, OH), 4.14 (2H, t, J = 5.9 Hz, CH_2), 4.17 (2H, s, CH_2), 5.52-5.57 (1H, m, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ -5.39, 18.13, 21.48, 25.86, 58.59,

62.36, 125.70, 139.04; **HRMS (**FTMS**)** calcd. for $C_{11}H_{25}O_2Si$ [M+H$^+$] 217.16183, found 217.16161.

(*Z*)-*tert*-Butyl-dimethyl-[2-methyl-4-(tetrahydro-pyran-2-yloxy)-but-2-enyloxy]-silane (33). Under nitrogen atmosphere to a solution of alcohol **28** (217 mg, 1 mmol) in CH_2Cl_2 (6 mL) at room temperature was added 3,4-dihydro-2H-pyran (126 mg, 1.5 mmol) in of CH_2Cl_2 (1 mL), and PPTS (25 mg, 0.1 mmol) in CH_2Cl_2 (1 mL). After stirring overnight, the solution was washed with saturated $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3x10 mL). The combined organic extracts were dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 10% ethyl acetate) to give 295 mg (98%) of **33** as colorless oil. R_f 0.87 (hexanes:ethyl acetate 1:1); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 0.07 (6H, s, $2CH_3$), 0.90 (9H, s, $3CH_3$), 1.49-1.85 (9H, m, CH_3, $3CH_2$), 3.47-3.54 (1H, m, CH), 3.83-3.91 (1H, m, CH), 3.99-4.06 (1H, s, CH), 4.18 (2H, s, CH_2), 4.21-4.28 (1H, m, CH), 4.61 (1H, t, J = 2.9 Hz, CH), 5.42 (1H, m, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ -5.33, 18.33, 19.52, 21.04, 25.45, 25.88, 30.64, 61.78, 62.22, 62.87, 97.91, 122.37, 139.68; **HRMS (**MALDI**)** calcd. for $C_{16}H_{32}O_3SiNa$ [M+Na$^+$] 323.2013, found 323.2001.

(*Z*)-2-Methyl-4-(tetrahydro-pyran-2-yloxy)-but-2-en-1-ol (34). A solution of 1 M TBAF in THF (4.36 mL, 4.36 mmol) was added dropwise to solution of **33** (437 mg, 1.45 mmol) in THF (15 mL) at 0 ^{o}C. The temperature was allowed to rise to room temperature. After stirring for 3 h, the reaction was quenched with brine (15 mL). The layers were separated, and the aqueous layer was extracted with ether (3x10 mL). The combined organic extracts were dried over Na_2SO_4. The solvent was removed at reduced

pressure, the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 30% ethyl acetate) to give 315 mg (94%) of **34** as colorless oil. R_f 0.25 (hexanes:ethyl acetate 1:1); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.52-1.86 (9H, m, CH_3, $3CH_2$), 2.59 (1H, s broad, OH), 3.50-3.57 (1H, m, CH), 3.82-3.90 (1H, m, CH), 4.00-4.27 (4H, s, $2CH_2$), 4.70 (1H, t, J = 2.9 Hz, CH), 5.51 (1H, t, J = 7.2 Hz, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 19.14, 21.85, 25.29, 30.38, 61.42, 61.96, 62.42, 96.98, 123.25, 141.01; **HRMS (**MALDI**)** calcd. for $C_{10}H_{18}O_3Na$ [M+Na^+] 209.1148, found 209.1158.

(*Z*)-2-[2-Methyl-4-(tetrahydro-pyran-2-yloxy)-but-2-enyl]-isoindole-1,3-dione (35). A solution of DIAD (223 mg, 1.1 mmol) in THF (1 mL) was added dropwise via a syringe to a solution of an alcohol **34** (187 mg, 1 mmol), phthalimide (162 mg, 1.1 mmol), and PPh_3 (289 mg, 1.1 mmol), in THF (4 mL) at the room temperature. After stirring overnight the solvent was removed at reduced pressure. The residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 25% ethyl acetate) to give 241 mg (76%) of **35** as white solid. R_f 0.63 (hexanes:ethyl acetate 1:1); mp = 77-78 ^{o}C; **^{1}H NMR** (CD_2Cl_2, 300 MHz) δ1.1.48-1.83 (9H, m, CH_3, $3CH_2$), 3.47-3.54 (1H, m, CH), 3.83-3.91 (1H, m, CH), 4.21-4.28 (1H, m, CH), 4.32 (2H, s, CH_2), 4.38-4.45 (1H, m, CH), 4.65-4.67 (1H, m, CH), 5.53-5.59 (1H, m, CH), 7.70-7.76 (2H, m, 2CH), 7.80-7.84 (2H, m, 2CH); **^{13}C NMR** (CD_2Cl_2, 75 MHz) δ 20.13, 21.85, 26.10, 31.25, 38.76, 62.65, 63.51, 98.55, 123.63, 126.70, 132.62, 134.30, 134.51, 168.66; **HRMS (**MALDI**)** calcd. for $C_{18}H_{21}NO_4Na$ [M+Na^+] 338.1363, found 338.1357.

(*Z*)-2-Methyl-4-(tetrahydro-pyran-2-yloxy)-but-2-enylamine (36). To a solution of phthalimide **35** (318 mg, 1 mmol) in EtOH (9 mL) was added hydrazine hydrate (150 mg, 3 mmol) in EtOH (1 mL). The solution was warmed up to 50 ^{0}C and

stirred at that temperature for 30 min, then refluxed for 2 h. The solution was cooled, filtrated, and the filter cake was washed with EtOH (3x10 mL). The solvent was removed at reduced pressure. A residue was redissolved in ether (20 mL). Then 1M NaOH (5 mL) was added to adjust the pH to 12. The aqueous layer was saturated with NaCl and extracted with ether (3x20 mL). The combined organic extracts were dried over Na_2SO_4. The solvent was removed at reduced pressure to give 144 mg (77%) of **36** as yellow liquid. This material was used without further purification. **^{1}H NMR** (CD_2Cl_2, 300 MHz) δ1.1.40-1.81 (11H, m, NH_2, CH_3, $3CH_2$), 3.23 (2H, s, CH_2), 3.44-3.50 (1H, m, CH), 3.78-3.86 (1H, m, CH), 3.95-4.02 (1H, m, CH), 4.15-4.21 (1H, m, CH), 4.57-4.60 (1H, m, CH), 5.34-5.41 (1H, m, CH); **^{13}C NMR** (CD_2Cl_2, 75 MHz) δ 20.09, 22.06, 26.07, 31.23, 42.91, 62.56, 63.14, 98.18, 122.61, 142.44.

(*Z*)-[2-Methyl-4-(tetrahydro-pyran-2-yloxy)-but-2-enyl]-carbamic acid *tert*-butyl ester (37). Amine **36** (600 mg, 3.24 mmol) was dissolved in iPrOH/H_2O (3:1, v/v, 140 mL) and solid Na_2CO_3 (3.24 g) was added. The solution was cooled to 0 ^{o}C. A solution of di-*tert*-butyl dicarbonate (1.46 g, 6.48 mmol) in iPrOH/H_2O (3:1, v/v, 20 mL) was added. The mixture was stirred overnight at room temperature. iPrOH was removed at reduced pressure, and the aqueous layer was extracted with ether (3x30 mL). The combined organic extracts were dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 15% ethyl acetate) to give 640 mg (69%) of **37** as colorless oil. R_f 0.56 (hexanes:ethyl acetate 1:1); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.26-1.89 (18H, m, $4CH_3$, $3CH_2$), 3.49-3.56 (1H, m, CH), 3.78 (2H, d, J = 5.9 Hz, CH_2), 3.83-3.90 (1H, m, CH), 4.00-4.07 (1H, m, CH), 4.21-4.27 (1H, m, CH), 4.63-4.65 (1H, m, CH), 4.75

(1H, s broad, NH), 5.51 (1H, t, J = 7 Hz, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 19.40, 22.03, 25.34, 28.34, 30.51, 40.81, 62.18, 62.71, 79.12, 97.74, 124.13, 138.00, 155.98; **HRMS (**MALDI**)** calcd. for $C_{15}H_{27}NO_4Na$ [M+Na^+] 308.1828, found 308.1828.

(*Z*)-(4-Hydroxy-2-methyl-but-2-enyl)-carbamic acid *tert*-butyl ester (38). PPTS (25 mg, 0.1 mmol) was added to the solution of acetal **37** (286 mg, 1 mmol) in EtOH (10 mL). The resulted solution was stirred at 55 ^oC for 1 h. Then solution was cooled down to room temperature. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 40% ethyl acetate) to give 198 mg (98%) of **38** as colorless oil. R_f 0.56 (hexanes:ethyl acetate 1:1); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.43 (9H, s, $3CH_3$), 1.76 (3H, t, J = 0.7 Hz, CH_3), 3.19 (1H, broad s, OH), 3.74 (2H, dd, J_1 = 5.6 Hz, J_2 = 0.7 Hz, CH_2), 4.14 (2H, t, J = 6.5 Hz, CH_2), 4.91 (1H, broad s, NH), 5.67 (1H, t, J = 7.3 Hz, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 21.62, 28.34, 40.67, 57.53, 79.88, 127.16, 136.24, 156.23; **HRMS (**MALDI**)** calcd. for $C_{10}H_{19}NO_3Na$ [M+Na^+] 224.1263, found 224.1268.

(*Z*)-[4-(Bis-benzyloxy-phosphoryloxy)-2-methyl-but-2-enyl]-carbamic acid *tert*-butyl ester (39). A solution of I_2 (609 mg, 2.4 mmol) in CH_2Cl_2 (30 mL) was added to a solution of $P(OBn)_3$ in CH_2Cl_2 (20 mL) at -40 ^oC. The mixture was stirred for 15 min and the cold bath was removed. After 30 min the solution became colorless, and then it was cannulated to a mixture of alcohol **38** (200 mg, 1.2 mmol) and pyridine (570 mg, 7.2 mmol) in CH_2Cl_2 (30 mL) at -40 ^oC over 30 min period. After the addition was complete, the mixture was stirred for 1 h at room temperature. Then the solvents were removed at reduced pressure, and the residue was redissolved in ether (50 mL) and washed with 0.3 M $KHSO_4$ (30 mL), $NaHCO_3$ sat. (30 mL), and brine (30 mL). The combined organic

extracts were dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 30% ethyl acetate) to give 320 mg (70%) of **39** as colorless oil. R_f 0.48 (hexanes:ethyl acetate 1:1); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.44 (9H, s, $3CH_3$), 1.74 (3H, t, J = 0.6 Hz, CH_3), 3.71 (2H, d, J = 6.2 Hz, CH_2), 4.54 (2H, d, J_1 = 7.7 Hz, J_2 = 2.3 Hz, CH_2), 4.94 (1H, broad s, NH), 5.02 (2H, dd, J_1 = 6.5 Hz, J_2 = 1.7 Hz, CH_2), 5.43 (1H, t, J = 7 Hz, CH), 7.35 (10H, s, 10CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 21.80, 28.39, 40.35, 63.37, 63.44, 69.14, 69.22, 121.83, 121.90, 127.93, 128.01, 128.52, 128.57, 128.69, 135.77, 135.87, 140.53, 156.09; **^{31}P NMR** ($CDCl_3$, 125 MHz) δ 0.68; **HRMS (**MALDI**)** calcd. for $C_{24}H_{32}NO_6PNa$ [M+Na^+] 484.1865, found 484.1872.

[4-(Bis-benzyloxy-phosphoryloxy)-2*R*,3*S*-dihydroxy-2-methyl-butyl]-carbamic acid *tert*-butyl ester (40). Olefine **39** (250 mg, 0.56 mmol) was asymmetrically dihydroxylated following the general Sharpless procedure. The residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 30% ethyl acetate) to give 172 mg (64%) of **40** as colorless oil. R_f 0.25 (hexanes:ethyl acetate 1:1); $[\alpha]_D^{20}$ + 11.7 (*c* 0.75, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ .04 (3H, s, CH_3), 1.43 (9H, s, $3CH_3$), 2.96-3.05 (2H, m, 2CH), 3.33-3.40 (1H, m, CH), 3.68-3.72 (1H, m, CH), 3.98-4.07 (1H, m, CH), 4.33 (1H, t, J = 10.5 Hz, CH), 4.69 (2H, s, CH_2), 4.98-5.09 (4H, m, $2CH_2$), 5.36 (1H, broad s, NH), 7.34 (10H, s, 10CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 19.67, 28.30, 48.40, 69.30, 69.37, 69.59, 69.67, 73.27, 73.80, 80.36, 128.05, 128.08, 128.62 135.52, 135.61, 158.32; **^{31}P NMR** ($CDCl_3$, 125 MHz) δ 0.72; **HRMS (**MALDI**)** calcd. for $C_{24}H_{34}NO_8PNa$ [M+Na^+] 518.1909, found 518.1909.

Phosphoric acid mono-(4-amino-2*R*,3*S*-dihydroxy-3-methyl-butyl) ester (42). Ester **41** (100 mg, 0.2 mmol) was dissolved in CH_3OH (5 mL), flushed with H_2, and Pd/C (8 mg) was added. The reaction was stirred for 1 h under a balloon of H_2 gas. The mixture was concentrated at reduced pressure. A ^1H NMR spectrum showed that no benzyl groups remained. The residue was redissolved in CH_3OH (4 mL) and 3 M HCl (1 mL) was added. After 30 min the mixture was concentrated at reduced pressure, and chromatographed on silica eluted with iPrOH:H_2O:NH_4OH (6:0.5:2.5 v/v/v) mixture to give 41 mg (89%) of **42** as white solid. R_f 0.38 (iPrOH:H_2O:NH_4OH (6:1:3 v/v/v)); $[\alpha]_D^{20}$ + 15.4 (*c* 0.65, D_2O); **^{1}H NMR** (D_2O, 300 MHz) δ 1.26 (3H, s, CH_3), 3.07 (1H, d, J = 13.2 Hz, CH), 3.25 (1H, d, J = 13.2 Hz, CH), 3.75-3.77 (1H, m, CH), 3.81-3.91 (1H, m, CH), 3.97-4.04 (1H, m, CH); **^{13}C NMR** (D_2O, 75 MHz) δ 20.39, 46.23, 64.51, 71.39, 75.78 (J = 7 Hz); **^{31}P NMR** (D_2O, 125 MHz) δ 4.20; **HRMS** (MALDI) calcd. for $C_5H_{13}NO_6P$ [M-H] 214.0486, found 214.0489.

4-(*tert*-Butyl-dimethyl-silanyloxy)-2*S*,3*S*-dihydroxy-3-methyl-butyric acid methyl ester (43). Olefine **27** (300 mg, 1.16 mmol) was asymmetrically dihydroxylated following the general Sharpless procedure. The residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 15% ethyl acetate) to give 250 mg (73%) of **43** as colorless oil. R_f 0.14 (hexanes:ethyl acetate 4:1); *er* 18/1 (by ^{19}F NMR analysis of the Mosher ester); $[\alpha]_D^{20}$ + 26.2 (*c* 1.8, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 0.09 (6H, s, $2CH_3$), 0.91 (9H, s, $3CH_3$), 1.17 (3H, d, J = 0.6 Hz, CH_3), 3.10 (1H, s, OH), 3.34-3.37 (1H, m, OH), 3.48 (1H, d, J = 10 Hz, CH), 3.68 (1H, d, J = 10 Hz, CH), 3.81 (3H, s, CH_3), 4.11 (1H, d, J = 8 Hz, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ -5.63, -5.58,

18.25, 19.64, 25.78, 52.45, 67.98, 73.26, 74.95, 173.65; **HRMS** (MALDI) calcd. for $C_{12}H_{26}O_5SiNa$ [M+Na^+] 301.1435, found 301.1435.

5-(*tert*-Butyl-dimethyl-silanyloxymethyl)-2*S*-methoxy-5-methyl-[1,3*S*]dioxolane-4-carboxylic acid methyl ester (44). To diol **43** (200 mg, 0.69 mmol) in CH_2Cl_2 (8mL) was added trimethyl orthoformate (291 mg, 2.74 mmol) in CH_2Cl_2 (1 mL) and CSA (16 mg, 0.07 mmol) in CH_2Cl_2 (1 mL). After stirring overnight, the solution was washed with saturated $NaHCO_3$. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3x20 mL). The combined organic extracts were dried over Na_2SO_4. The solvent was removed at reduced pressure to give 230 mg (quant) of **44** as colorless oil. This material was unstable on silica column and was used without purification. R_f 0.63 (hexanes:ethyl acetate 3:2); *dr* 87/13 (by ^{1}H NMR analysis of the orthoester); for major diastereomer **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 0.02 (6H, s, $2CH_3$), 0.86 (9H, s, $3CH_3$), 1.49 (3H, s, CH_3), 3.34 (3H, s, CH_3), 3.56 (2H, dd, J_1 = 18 Hz, J_2 = 10.6 Hz, CH_2), 4.40 (1H, s, CH), 5.88 (1H, s, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ -5.66, -5.55, 18.25, 22.43, 25.72, 51.56, 52.13, 65.56, 79.04, 84.66, 115.95, 168.47; **HRMS** (MALDI) calcd. for $C_{14}H_{28}O_6SiNa$ [M+Na^+] 343.1547, found 343.1558.

[5-(*tert*-Butyl-dimethyl-silanyloxymethyl)-2*R*-methoxy-5-methyl-[1,3*S*]dioxolan-4-yl]-methanol (45). A solution of 2 M $LiBH_4$ in THF (0.6 mL, 1.2 mmol) was added dropwise by a syringe to a solution of **44** (200 mg, 0.6 mmol) in ether (20 mL) at room temperature. The resulting solution was stirred for 1 h at the same temperature. After addition of EtOH (5 mL) and brine (5 mL), the organic layer was separated, and the aqueous layer was extracted ether (3x10 mL). The combined organic extracts were dried over Na_2SO_4. The solvent was removed at reduced pressure to give

175 mg (quant) of **45** as colorless oil. This material was unstable on silica column and was used without purification. R_f 0.63 (hexanes:ethyl acetate 3:2); *dr* 87/13 (by ^{1}H NMR analysis of the orthoester); for major diastereomer **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 0.11 (6H, s, $2CH_3$), 0.91 (9H, s, $3CH_3$), 1.45 (3H, s, CH_3), 3.32 (3H, s, CH_3), 3.71-4.09 (5H, m, CH, $2CH_2$), 5.67 (1H, s, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ -5.72, -5.70, 18.07, 22.79, 25.68, 51.72, 60.33, 64.95, 81.26, 82.63, 114.79; **HRMS** (FTMS) calcd. for $C_{12}H_{25}O_4Si$ [M-OCH_3] 261.1517, found 261.15164.

Toluene-4-sulfonic acid 5-(*tert*-butyl-dimethyl-silanyloxymethyl)-2*R*-methoxy-5-methyl-[1,3*S*]dioxolan-4-ylmethyl ester (46). Alcohol **45** (60 mg, 0.21 mmol) and DMAP (75 mg, 0.62 mmol) were dissolved in CH_2Cl_2 (5 mL). To the solution was added *p*-TsCl (78 mg, 0.41 mmol) in CH_2Cl_2 (2 mL) via syringe. The mixture was allowed to stir overnight. The solvents were removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 10% ethyl acetate) to give 90 mg (98%) of **46** as colorless oil. R_f 0.59 (hexanes:ethyl acetate 3:2); *dr* 87/13 (by ^{1}H NMR analysis of the orthoester); $[\alpha]_D^{20}$ + 10.2 (*c* 0.9, $CHCl_3$); for major diastereomer **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 0.82 (9H, s, $3CH_3$), 1.34 (3H, s, CH_3), 2.45 (3H, s, CH_3), 3.29 (3H, s, CH_3), 3.32 (1H, d, J = 10.5 Hz, CH), 3.50 (1H, d, J = 10.5 Hz, CH), 4.10-4.18 (2H, m, CH_2), 4.33-4.41 (1H, m, CH), 5.68 (1H, s, CH), 7.13 (2H, d, J = 8 Hz, 2CH), 7.80 (2H, d, J = 8.3 Hz, 2CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ -5.85, -5.74, 17.88, 21.59, 25.63, 51.45, 65.08, 68.35, 79.73, 82.52, 115.35, 128.00, 129.86, 132.74, 144.89; **HRMS** (FTMS) calcd. for $C_{19}H_{31}O_6SSi$ [M-OCH_3] 415.1605, found 415.16047.

Toluene-4-sulfonic acid 4-(*tert*-butyl-dimethyl-silanyloxy)-2*R*,3*S*-dihydroxy-3-methyl-butyl ester (48). To **46** (230 mg, 0.54 mmol) was added HCl in THF (6 mL, 3M HCl:THF, 1:25). After stirring for 30 min, the solution was cooled to 0 ^{o}C and 6 mL of half saturated ammonia in MeOH were added. The solution was stirred for 15 min at room temperature. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 30% ethyl acetate) to give 160 mg (76%) of **48** as colorless oil. R_f 0.36 (hexanes:ethyl acetate 1:1); $[\alpha]_D^{20}$ + 18.9 (*c* 1.7, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 0.06 (6H, s, $2CH_3$), 0.88 (9H, s, $3CH_3$), 1.07 (3H, s, CH_3), 2.45 (3H, d, J = 0.3 Hz, CH_3), 2.71-2.74 (2H, broad, 2OH), 3.38 (1H, d, J = 9.8 Hz, CH), 3.67 (1H, d, J = 9.8 Hz, CH), 3.82 (1H, dd, J_1 = 5.8 Hz, J_2 = 2.5 Hz, CH), 4.09 (1H, dd, J_1 = 8.2 Hz, J_2 = 2.0 Hz, CH), 4.32 (1H, dd, J_1 = 8.0 Hz, J_2 = 2.5 Hz, CH), 7.36 (2H, d, J = 8 Hz, 2CH), 7.81 (2H, d, J = 8.2 Hz, 2CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ -5.62, 18.13, 18.99, 21.63, 25.77, 67.55, 71.99, 72.65, 72.94, 127.97, 129.92, 132.60, 145.00; **HRMS** (FTMS) calcd. for $C_{18}H_{33}O_6SSi$ [M+H$^+$] 405.17616, found 405.17603.

1-(*tert*-Butyl-dimethyl-silanyloxy)-2-oxiranyl-propan-2-ol (49). To diol **48** (367 mg, 0.91 mmol) in THF (6 mL) at 0 ^{o}C was added *t*BuOK (112 mg, 1.0 mmol) in THF (4 mL). The solution was stirred for 1 h at 0 ^{o}C. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate to give 196 mg (93%) of **49** as colorless oil. R_f 0.36 (hexanes:ethyl acetate 1:1); $[\alpha]_D^{20}$ + 15.0 (*c* 2.6, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 0.08 (6H, s, $2CH_3$), 0.91 (9H, s, $3CH_3$), 1.18 (3H, s, CH_3), 2.36 (1H, s, OH), 2.74 (1H, dd, J_1 = 4.0 Hz, J_2 = 1.0 Hz, CH), 2.83 (1H, dd, J_1 = 2.8 Hz, J_2 = 2.2 Hz, CH), 3.01 (1H, dd, J_1 = 2.8

Hz, J_2 = 1.2 Hz, CH), 3.51 (1H, d, J = 9.8 Hz, CH), 3.61 (1H, d, J = 9.8 Hz, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ -5.56, 18.20, 20.92, 25.78, 44.04, 55.68, 68.42, 70.23; **HRMS** (FTMS) calcd. for $C_{11}H_{25}O_3Si$ [M+H^+] 233.15675, found 233.15712.

2-Oxiranyl-propane-1,2-diol (50). Et_3N-3HF complex (1.1 g, 6.9 mmol) in THF (2 mL) was added dropwise to a solution of silyl ether **49** (160 mg, 0.69 mmol) in THF (8 mL) at room temperature. After stirring overnight, Et_3N (10 mL) was added. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 30% ethyl acetate) to give 68 mg (83%) of **50** as colorless oil. R_f 0.29 (hexanes:ethyl acetate 1:1); *er* 17/1 (by ^{19}F NMR analysis of the Mosher ester); $[\alpha]_D^{20}$ + 17.4 (*c* 0.65, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.26 (3H, s, CH_3), 2.36 (1H, m, OH), 2.50 (1H, s, OH), 2.81-2.88 (2H, m, CH_2), 3.04 (1H, dd, J_1 = 2.9 Hz, J_2 = 1.1 Hz, CH), 3.49-3.62 (2H, m, CH_2); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 22.28, 44.49, 56.55, 67.21, 70.29; **HRMS** (FTMS) calcd. for $C_5H_9O_2$ [M-OH] 101.05971, found 101.06000.

Thiophosphoric acid S-(2,3,4-trihydroxy-3-methyl-butyl) ester (51). To epoxide **50** (34 mg, 0.29 mmol) was added trisodium thiophosphate (126 mg, 0.32 mmol) in H_2O (0.6 mL) at room temperature. The solution was stirred overnight. After that the mixture was lyophilized and the residue was chromatographed on cellulose column eluted with (50 mM NH_4HCO_3 iPrOH:H_2O) using gradient method (iPrOH:H_2O from 8:2 to 7:3 v/v) to give 25 mg of **51** (32%). *Rf.* 0.42 (iPrOH:H_2O:NH_4OH 6/1/3 v/v/v) $[\alpha]_D^{20}$ + 16.3 (*c* 0.53, D_2O); **^{1}H NMR** (D_2O, 300 MHz) δ 1.09 (3H, s, CH_3), 2.67-2.78 (1H, m, CH), 2.96-3.05 (1H, m, CH), 3.51 (2H, dd, J_1 = 17.2 Hz, J_2 = 11.8 Hz, CH_2), 3.72 (1H, dd, J_1 = 8.4 Hz, J_2 = 2.2 Hz, CH_2); **^{13}C NMR** (D_2O, 75 MHz) δ 17.70, 31.53 (J = 3

Hz), 66.70, 75.02, 75.48 (J = 3 Hz); **^{31}P NMR** (D_2O, 125 MHz) δ 17.82; **HRMS** (FTMS) calcd. for $C_5H_{14}O_6PS$ [M+H^+] 233.02432, found 233.02482.

***tert*-Butyl(3-methylbut-2-enyloxy)diphenylsilane (52)**. To the mixture of the 3-methylbut-2-en-1-ol (15 g, 172.4 mmol) and DMAP (32 g, 258.6 mmol) in CH_2Cl_2 (300 mL) was added a solution of TBDPSCl (53.2 g, 275 mmol) in CH_2Cl_2 (200 mL) via cannula in 30 min at room temperature. The resulting mixture was stirred overnight, transferred into a separation funnel containing brine (500 mL), and extracted with CH_2Cl_2 (3x100 mL). The combined extracts were dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 5% ethyl acetate) to give 52 g (92%) of **52** as colorless oil. R_f 0.63 (hexanes:ethyl acetate 7:3); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.05 (9H, s, $3CH_3$), 1.45 (3H, s, CH_3), 1.69 (3H, J = 1 Hz, CH_3), 4.20 (2H, dt, J_1 = 6.5 Hz, J_2 = 1 Hz, CH_2), 5.35-5.41 (1H, m, CH), 7.34-7.43 (6H, m, 6CH), 7.68-7.72 (4H, m, 4CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 17.91, 19.16, 25.70, 26.83, 61.10, 124.18, 127.56, 129.47, 133.77, 134.03, 135.59; **HRMS** (FTMS) calcd. for $C_{21}H_{28}OSiNa$ [M+Na^+] 347.18016, found 347.18007.

(*E*)-4-(*tert*-Butyldiphenylsilyloxy)-2-methylbut-2-enal (53) and (*E*)-4-(*tert*-butyldiphenylsilyloxy)-2-methylbut-2-en-1-ol (54). To a solution of **52** (25.81 g, 79.53 mmol) in CH_2Cl_2 (100 mL) was added TBHP (30 mL, 90% solution in water), selenious acid (1 g), and salicylic acid (1 g). The mixture was stirred for 17 h at room temperature. The solution was transferred into a separation funnel containing water (200 mL) and was extracted with ether (3x50 mL). The combined extracts were dried over Na_2SO_4. The solvent was removed at reduced pressure, and the TBHP was removed at reduced

pressure by co-evaporation with toluene (3x100 mL). The residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 25% ethyl acetate) to give 16.4 g (61%) of aldehyde **53** as colorless oil. R_f 0.5 (hexanes:ethyl acetate 7:3); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.05 (9H, s, $3CH_3$) 1.56 (3H, dd, J_1 = 2.5 Hz, J_2 = 1.2 Hz, CH_3), 4.56 (2H, dq, J_1 = 5.3 Hz, J_1 = 1 Hz, CH_2), 6.59-6.65 (1H, m, CH), 7.39-7.48 (6H, m, 6CH), 7.70-7.74 (4H, m, 4CH), 9.43 (1H, s, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 9.22, 19.06, 26.66, 61.20, 127.64, 127.80, 129.87, 132.89, 135.44, 137.80, 152.41, 194.46; **HRMS** (FTMS) calcd. for $C_{21}H_{27}O_2Si$ [M+H$^+$] 339.17748, found 339.17740. and 8.4 g (31%) of alcohol **54** as colorless oil. R_f 0.25 (hexanes:ethyl acetate 7:3); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.05 (9H, s, $3CH_3$), 1.41 (1H, s broad, OH), 1.48 (3H, s, CH_3), 3.95 (2H, d, J = 4.7 Hz, CH_2), 4.27 (2H, dd, J_1 = 6.2 Hz, J_2 = 1 Hz, CH_2), 5.59-5.65 (1H, m, CH), 7.35-7.46 (6H, m, 6CH), 7.67-7.71 (4H, m, 4CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 13.73, 19.11, 26.78, 60.72, 68.18, 124.84, 127.61, 129.57, 133.79, 135.57, 136.13; **HRMS** (MALDI) calcd. for $C_{21}H_{28}O_2SiNa$ [M+Na$^+$] 363.1751, found 363.1741.

(*E*)-4-(*tert*-Butyldiphenylsilyloxy)-2-methylbut-2-en-1-ol (54). To a solution of aldehyde **53** (14.84 g, 43.84 mmol) in EtOH (200 mL) at 0 ^{o}C was added $NaBH_4$ (6.6 g, 175.4 mmol) in small portions during 15 min. The mixture was stirred for 2 h at room temperature. The solvent was removed at reduced pressure. The residue was portioned between ether (100 mL) and water (400 mL). The aqueous layer was extracted with ether (3x100 mL). The combined organic layers were washed with brine (100 mL), and dried over Na_2SO_4. After evaporation of the solvents, the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 25% ethyl acetate) to give 12.5 g (84%) of **54** as colorless oil. R_f 0.25 (hexanes:ethyl acetate 7:3); **^{1}H NMR** ($CDCl_3$,

300 MHz) δ 1.05 (9H, s, $3CH_3$), 1.41 (1H, s broad, OH), 1.48 (3H, s, CH_3), 3.95 (2H, d, J = 4.7 Hz, CH_2), 4.27 (2H, dd, J_1 = 6.2 Hz, J_2 = 1 Hz, CH_2), 5.59-5.65 (1H, m, CH), 7.35-7.46 (6H, m, 6CH), 7.67-7.71 (4H, m, 4CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 13.73, 19.11, 26.78, 60.72, 68.18, 124.84, 127.61, 129.57, 133.79, 135.57, 136.13; **HRMS** (MALDI) calcd. for $C_{21}H_{28}O_2SiNa$ [M+Na^+] 363.1741, found 363.1751.

((2*R*,3*R*)-3-((*tert*-Butyldiphenylsilyloxy)methyl)-2-methyloxiran-2-yl) methanol (55). An oven dried 250 mL round-bottomed flask equipped with a magnetic stirring bar was charged with 3.0 g of 4Å molecular sieves and CH_2Cl_2 (100 mL). The mixture was cooled to -30 ^oC and D-(-)-diethyl tartrate (1.8 g, 8.46 mmol) and $Ti(OiPr)_4$, (2 g, 7.05 mmol, via) were added with stirring. The reaction mixture was stirred at -30 ^oC as TBHP (9.4 mL, 47 mmol, 5-6 M in dodecane) was added by syringe. The resulting mixture was stirred at -30 ^oC for 30 min. Alcohol **54** (8 g, 23.5 mmol) in CH_2Cl_2 (50 mL), was added dropwise via cannula over 20 min. The mixture was stirred for 5 h at -20 to -15 ^oC. The cold bath was removed and a 10% NaOH solution in brine (20 mL) was added. The mixture was allowed to stir for additional 30 min., before the contents of the flask were transferred to a separation funnel and organic layer was removed. The aqueous layer was extracted with ether (3x20 mL). The combined organic layers were dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 30% ethyl acetate) to give 7.95 g (95%) of **55** as colorless oil. R_f 0.23 (hexanes:ethyl acetate 7:3); *er* 12/1 (by ^{19}F NMR analysis of the Mosher ester); $[\alpha]_D^{20}$ + 10.8 (*c* 0.9, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.06 (9H, s, $3CH_3$), 1.12 (3H, s, CH_3), 2.07 (1H, dd, J_1 = 8.4 Hz, J_2 = 5 Hz, OH), 3.33 (1H, t, J =5.4 Hz, CH), 3.52-3.70 (2H, m, CH_2), 3.80-3.90 (2H,

m, CH_2), 7.38-7.49 (6H, m, 6CH), 7.69-7.77 (4H, m, 4CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 14.08, 19.13, 26.68, 59.68, 60.68, 62.39, 65.01, 127.71, 129.75, 133.03, 133.27, 135.49, 135.53; **HRMS** (MALDI) calcd. for $C_{21}H_{28}O_3SiNa$ [M+Na^+] 379.1700, found 379.1710.

(((2*R*,3*S*)-3-(Bromomethyl)-3-methyloxiran-2-yl)methoxy)(*tert*-butyl) diphenylsilane (56). To a solution of epoxy alcohol **55** (1.5 g, 4.2 mmol) and CBr_4 (1.7 g, 5.0 mmol) in CH_2Cl_2 (40 mL) at 0 ^oC was added a solution of PPh_3 (1.45 g, 5.46 mmol) in CH_2Cl_2 (20 mL). The resulting solution was stirred at 0 ^oC for 2 h and diluted with ether (60 mL), and the white precipitate was removed by filtration. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 10% ethyl acetate) to give 1.5 g (85%) of **56** as colorless oil. R_f 0.69 (hexanes:ethyl acetate 4:1); $[\alpha]_D^{20}$ - 0.5 (*c* 0.785, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.07 (9H, s, $3CH_3$), 1.29 (3H, s, CH_3), 3.15 (1H, t, J =5.4 Hz, CH), 3.30 (2H, dd, J_1 = 30 Hz, J_2 = 10 Hz, CH_2), 3.73-3.85 (2H, m, CH_2), 7.36-7.46 (6H, m, 6CH), 7.67-7.70 (4H, m, 4CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 15.15, 19.15, 2.69, 39.12, 59.29, 62.43, 64.23, 127.76, 129.82, 132.96, 133.18, 135.51 135.54; **HRMS** (MALDI) calcd. for $C_{21}H_{27}BrO_2SiNa$ [M+Na^+] 441.0856, found 441.0858.

***(S)*-((2*S*,3*R*)-3-((*tert*-Butyldiphenylsilyloxy)methyl)-2-methyloxiran-2-yl) methyl ethanethioate (57)**. To a solution of KSAc (826 mg, 7.24 mmol) in DMF (40 mL) was added via cannula a solution of epoxy bromide **56** (1.5 g, 3.62 mmol) in DMF (20 mL) at room temperature. The solution was stirred overnight at room temperature, and transferred to a separation funnel containing water (600 mL) and ether (100 mL). The aqueous layer was extracted with ether (3x100 mL). The combined organic layers were dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was

chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 10% ethyl acetate) to give 1.17 g (79%) of **57** as colorless oil. R_f 0.68 (hexanes:ethyl acetate 4:1); $[\alpha]_D^{20}$ + 15.4 (*c* 0.8, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.06 (9H, s, $3CH_3$), 1.15 (3H, s, CH_3), 2.36 (3H, s, CH_3), 3.08 (2H, d, J = 14 Hz, CH_2), 3.13 (1H, t, J = 5.4 Hz, CH), 3.69-3.82 (2H, m, CH_2), 7.36-7.46 (6H, m, 6CH), 7.66-7.70 (4H, m, 4CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 16.02, 19.15, 26.68, 30.42, 36.67, 59.26, 62.40, 62.85, 127.73, 129.77, 133.04, 133.27, 135.51, 135.54, 194.51; **HRMS** (MALDI) calcd. for $C_{23}H_{30}O_3SSiNa$ [M+Na^+] 437.1577, found 437.1578.

(*R*)-2-(*tert*-Butyldiphenylsilyloxy)-1-((R)-2-methylthiiran-2-yl)ethanol (58). To the solution of thioacetate **57** (1.17 g, 2.82 mmol) in MeOH (20 mL) at -30 ^oC was added MeONa (25% wt, 153 mg, 0.71 mmol) in MeOH (10 mL) via syringe. The mixture was left overnight in the freezer at -20 ^oC without stirring. The reaction mixture was quenched with saturated solution of NH_4Cl (10 mL). The solvent was removed at reduced pressure, and the mixture was redissolved in ether (100 mL) and water (100 mL). The aqueous layer was extracted with ether (3x20 mL). The combined organic layers were dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 15% ethyl acetate) to give 1.17 g (95%) of **58** as colorless oil. R_f 0.6 (hexanes:ethyl acetate 4:1); *er* 10/1 (by ^{19}F NMR analysis of the Mosher ester); $[\alpha]_D^{20}$ + 5.5 (*c* 1.26, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.07 (9H, s, $3CH_3$), 1.57 (3H, s, CH_3), 2.34 (1H, d, J = 1.2 Hz, CH), 2.60 (1H, d, J = 1.1 Hz, CH), 2.75 (1H, d, J = 2.6 Hz, CH), 3.52-3.56 (1H, m, CH), 3.78-3.89 (2H, m, CH_2), 7.36-7.48 (6H, m, 6CH), 7.65-7.71 (4H, m, 4CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 19.17, 21.78, 26.79, 32.25, 46.57, 65.51, 76.24, 127.77,

127.79, 129.83, 132.82, 132.98, 135.53, 135.55; **HRMS** (MALDI) calcd. for $C_{21}H_{28}O_2SSiNa$ [M+Na^+] 395.1472, found 395.1456.

(*R*)-2-(*tert*-Butyldiphenylsilyloxy)-1-((R)-2-methylthiiran-2-yl)ethyl acetate (59). To a solution of alcohol **58** (1 g, 2.68 mmol) and DMAP (530 mg, 4.29 mmol) in CH_2Cl_2 (20 mL) was added a solution of AcCl (228 μL, 3.22 mmol) in CH_2Cl_2 (5 mL) by syringe over 15 min at room temperature. The solution was stirred overnight, and washed with water (20 mL). The aqueous layer was extracted with ether (3x10 mL). The combined organic layers were dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 10% ethyl acetate) to give 1.01 g (91%) of **58** as colorless oil. R_f 0.69 (hexanes:ethyl acetate 4:1); $[\alpha]_D^{20}$ + 2.7 (*c* 1.44, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.04 (9H, s, $3CH_3$), 1.51 (3H, s, CH_3), 2.07 (3H, s, CH_3), 2.25 (1H, d, J = 1.1 Hz, CH), 2.59 (1H, d, J = 1 Hz, CH), 3.88-3.96 (2H, m, CH_2), 4.77 (1H, dd, J_1 = 4.4 Hz, J_2 = 2 Hz, CH), 7.36-7.47 (6H, m, 6CH), 7.65-7.69 (4H, m, 4CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 19.13, 21.06, 21.65, 26.63, 32.87, 42.14, 64.28, 79.41, 127.70, 127.72, 129.72, 129.79, 133.02, 133.23, 135.53, 135.61, 170.22; **HRMS** (FTMS) calcd. for $C_{23}H_{30}O_3SSiNa$ [M+Na^+] 437.15765, found 437.15771.

(2*S*,3*R*)-2-(Acetylthio)-4-(*tert*-butyldiphenylsilyloxy)-2-methylbutane-1,3-diyl diacetate (60). Thiirane **59** (4g, 9.65 mmol) was dissolved in acetic anhydride (250 mL) and acetic acid (250 mL). To the resulting mixture was added AcOK (47.3 g, 482.5 mmol). The mixture was heated at 140 ^{o}C for 12 h. Acetic acid and acetic anhydride were removed at reduced pressure with co-evaporation with toluene (3x200 mL). The residue was dissolved in water (300 mL) and extracted with ether (3x100 mL). The combined

organic layers were dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 25% ethyl acetate) to give 1.55 g (31%) of **60** as colorless oil. R_f 0.59 (hexanes:ethyl acetate 1:1); $[\alpha]_D^{20}$ + 1.4 (*c* 1.75, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.02 (9H, s, $3CH_3$), 1.51 (3H, s, CH_3), 2.00 (3H, s, CH_3), 2.09 (3H, s, CH_3), 2.21 (3H, s, CH_3), 3.77-3.91 (2H, m, CH_2), 4.39 (2H, q, J = 10.4 Hz, CH_2), 5.55 (1H, dd, J_1 = 4 Hz, J_2 = 3.4 Hz, CH), 7.36-7.47 (6H, m, 6CH), 7.63-7.68 (4H, m, 4CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 18.73, 19.05, 20.75, 20.91, 26.57, 31.32, 54.90, 62.81, 65.47, 74.39, 127.73, 129.75, 129.81, 132.84, 133.04, 135.50, 135.62, 169.74, 170.42, 194.56; **HRMS** (FTMS) calcd. for $C_{27}H_{36}O_6SSiNa$ [M+Na^+] 539.1900, found 539.18958.

(2*S*,3*R*)-4-(*tert*-Butyldiphenylsilyloxy)-2-methyl-2-(pyridin-2-yldisulfanyl)butane-1,3-diol (61). To a solution of triacetate **60** (280 mg, 0.54 mmol) and 2,2'-pyridyl disulfide (358 mg, 1.63 mmol) in MeOH (10 mL) at -30 ^oC was added MeONa (25% wt, 90 mg, 0.41 mmol) in MeOH (1 mL) via syringe. The mixture was left overnight in freezer at -20 ^oC without stirring, and then quenched with a saturated solution of NH_4Cl (2 mL). The solvent was removed at reduced pressure, and the mixture was diluted with water (20 mL), ether (20 mL) and transferred to a separation funnel. The aqueous layer was extracted with ether (3x20 mL). The combined organic layers were dried over Na_2SO_4. The solvent was removed at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 25% ethyl acetate) to give 240 mg (89%) of **61** as colorless oil. R_f 0.2 (hexanes:ethyl acetate 4:1); $[\alpha]_D^{20}$ + 60.8 (*c* 1.0, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.07 (9H, s, $3CH_3$), 1.24 (3H, s, CH_3), 3.61-3.67 (1H, m, CH), 3.80-3.89 (3H, m, CH, CH_2), 4.00-4.07 (1H,

m, CH), 4.21 (1H, d, J = 4.7, CH), 5.99 (1H, t, J = 7.4 Hz, CH), 7.12-7.16 (1H, m, CH), 7.33-7.71 (12H, m, 12CH), 8.45-8.47 (1H, m, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 18.06, 19.17, 26.78, 59.09, 64.75, 65.21, 74.93, 121.58, 122.05, 127.78, 129.81, 132.82, 132.97, 135.53, 135.61, 136.88, 149.67, 158.78; **HRMS** (MALDI) calcd. for $C_{26}H_{34}NO_3S_2Si$ [M+H$^+$] 500.1744, found 500.1758.

(2*S*,3*R*)-4-(*tert*-Butyldiphenylsilyloxy)-2-methyl-2-(pyridin-2-yldisulfanyl) butane-1,3-diyl diacetate (62). To a solution of diol **61** (260 mg, 0.52 mmol) and DMAP (225 mg, 1.82 mmol) in CH_2Cl_2 (10 mL) was added a solution of AcCl (93 μL, 1.3 mmol) in CH_2Cl_2 (1 mL) by syringe at room temperature. The solution was stirred overnight, and washed with water (20 mL). The aqueous layer was extracted with ether (3x10 mL). The combined organic extracts were dried over Na_2SO_4. After the solvent was removed at reduced pressure, the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 15% ethyl acetate) to give 280 mg (92%) of **62** as colorless oil. R_f 0.69 (hexanes:ethyl acetate 4:1); $[\alpha]_D^{20}$ + 26.1 (*c* 1.4, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.03 (9H, s, $3CH_3$), 1.27 (3H, s, CH_3), 1.92 (3H, s, CH_3), 2.06 (3H, s, CH_3), 3.87 (1H, dd, J_1 = 7.4 Hz, J_2 = 3.9 Hz, CH), 4.04 (1H, dd, J_1 = 8.1 Hz, J_2 = 3.3 Hz, CH), 4.16 (2H, dd, J_1 = 23.8 Hz, J_2 = 11.8 Hz, CH_2), 5.42 (1H, dd, J_1 = 4.3 Hz, J_2 = 3.3 Hz, CH), 7.04-7.08 (1H, m, CH), 7.37-7.70 (12H, m, 12CH), 8.39-8.41 (1H, m, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 18.55, 19.05, 20.64, 20.90, 26.58, 54.84, 62.74, 66.74, 75.08, 120.14, 120.89, 127.74, 127.77, 129.76, 129.84, 132.85, 133.00, 135.51, 135.61, 136.80, 149.42, 160.05, 169.70, 170.34; **HRMS** (MALDI) calcd. for $C_{30}H_{38}NO_5S_2Si$ [M+H$^+$] 584.1955, found 584.1933.

(2S,3R)-4-hydroxy-2-methyl-2-(pyridin-2-yldisulfanyl)butane-1,3-diyl diacetate (63). To a solution of silyl ether **62** (200 mg, 0.34 mol) in THF (5 mL) was added 1 M solution TBAF in THF (1 mL, 1 mmol) at room temperature. After stirring for 2 h at room temperature the solution was concentrated at reduced pressure, and the residue was chromatographed on silica with gradient elution by hexanes/ethyl acetate (0% to 40% ethyl acetate) to give 280 mg (92%) of **62** as colorless oil. R_f 0.69 (hexanes:ethyl acetate 4:1); $[\alpha]_D^{20}$ + 62.3 (*c* 1.00, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.44 (3H, s, CH_3), 1.62 (1H, broad s, OH), 2.07 (3H, s, CH_3), 2.10 (3H, s, CH_3), 3.86 (1H, m, CH), 4.15 (1H, dd, J_1 = 7.8 Hz, J_2 = 3.7 Hz, CH), 4.38-4.44 (3H, m, CH, CH_2), 6.32 (1H, d, J = 5.2 Hz, CH) 7.16-7.20 (1H, m, CH), 7.42-7.45 (1H, m, CH), 7.58-7.64 (1H, m, CH) 8.49-8.52 (1H, m, CH); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 20.69, 20.82, 21.02, 58.11, 65.22, 65.73, 72.44, 121.77, 122.06, 137.08, 149.72, 158.53, 170.56, 171.09; **HRMS** (MALDI) calcd. for $C_{14}H_{20}NO_5S_2$ [M+H$^+$] 346.0777, found 346.0782.

References

1. Poulter, C. D.; Rilling, H. C. *Biosynthesis of Isoprenoid compounds*; Porter, J. W., Spurgeon, S. L., Eds.; Willey; New York, 1981; Vol. 1, pp 162-209.

2. (a) Corey, E. J.; Volante, R. R. *J. Am. Chem. Soc.*, **1976**, *98*, 1291-1291. (b) Cane, D. E.; Yang, G.; Xue, Q.; Shim, J. H. *Biochemistry*, **1995**, *34*, 2471-2479.

3. McClard, R. W.; Fujita, T. S.; StremLer, K. E.; Poulter, C. D. *J. Am. Chem. Soc.*, **1987**, *109*, 5544-5545.

4. Stremler, K. E.; Poulter, C. D. *J. Am. Chem. Soc.*, **1987**, *109*, 5542-5544.

5. (a) Phan, R. M.; Poulter, C. D. *Org. Lett.*, **2000**, *2*, 2287-2289. (b) Phan, R. M.; Poulter, C. D. *J. Org. Chem.*, **2001**, *66,* 6705-6710.

6. Rohdich, F.; Hecht, S.; Bacher, A.; Eisenreich, W. *Pure Appl. Chem.*, **2003**, *75*, 393–405.

7. Koppisch, A. T.; Blagg, B. S. J.; Poulter, C. D. *Org. Lett.,* **2000**, *2*, 215-217.

8. Corey, E. J.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.*, **1969**, *91*, 1851-1852.

9. Gefflaut, T.; Lemaire, M.; Valentin, M-L; Bolte, J. *J. Org. Chem.*, **1997**, *62*, 5920-5922.

10. Stamatov, S. D.; Stawinski, J. *Org. Biomol. Chem.*, **2007**, *5*, 3787–3800.

11. Mitsunobu, O. *Synthesis* **1981**, 1-28.

12. Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.,* **1994**, *94*, 2483-547.

13. Norrby, P.-O.; Kolb, H. C.; Sharpless, K. B. *J. Am. Chem. Soc.*, **1994***, 116,* 8410-8478.

14. Testa, C. A.; Lherbet, C.; Pojer, F.; Noel, J. P.; and Poulter, C. D. *Biochim Biophys Acta*, **2006**, *1764*, 85-96.

15. Lherbet, C.; Pojer, F.; Richard, S. B.; Noel, J. P.; Poulter, C. D. *Biochemistry*, **2006**, *45*, 3548-3553.

16. Cassera, M. B.; Gozzo, F. C.; D'Alexandri, F. L.; Merino, E. F.; del Portillo, H. A.; Peres, V. J.; Almeida, I. C.; Eberlin, M. N.; Wunderlich, G.; Wiesner, J.; Jomaa, H.; Kimura, E. A.; Katzin, A. M. *J. Biol. Chem*., **2004**, *279*, 51749-51759.

17. Gao, Y.; Klunder, J. M.; Hanson, R. M.; Masamune, H.; Ko, S. Y.; Sharpless, K. B. *J. Am. Chem. Soc.,* **1987**, *109*, 5765-5780.

18. Baughman, T. W.; Sworen, J. C.; Wagener, K. B. *Tetrahedron,* **2004**, *60*, 10943-10948.

19. Payne, G. B. *J. Org. Chem.*, **1962**, *27*, 3819-3822.

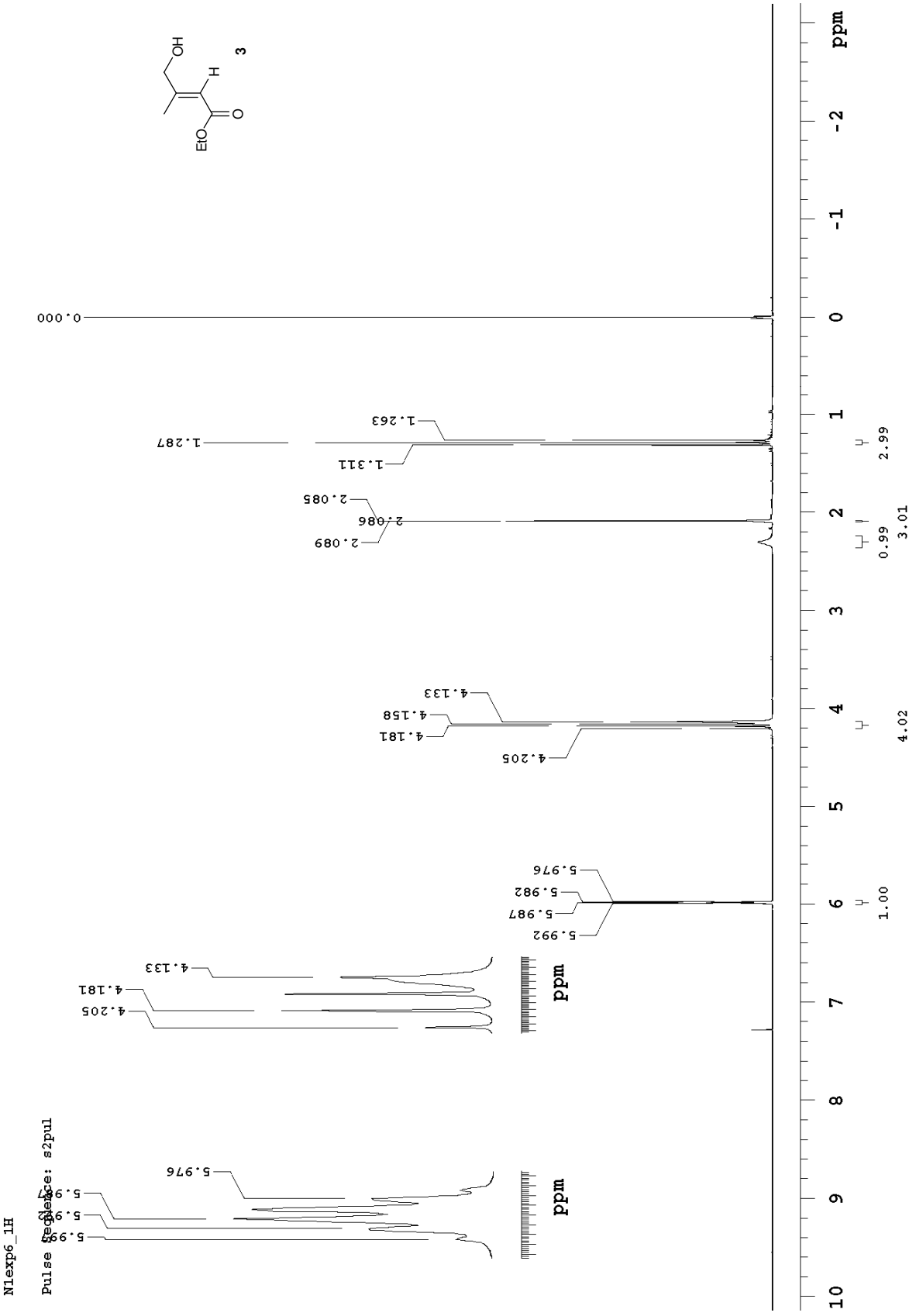
20. Brånalt, J.; Kvarnstrom, I.; Classon, B.; Samuelsson, B. *J. Org. Chem.* **1996**, *61*, 3604-3610.

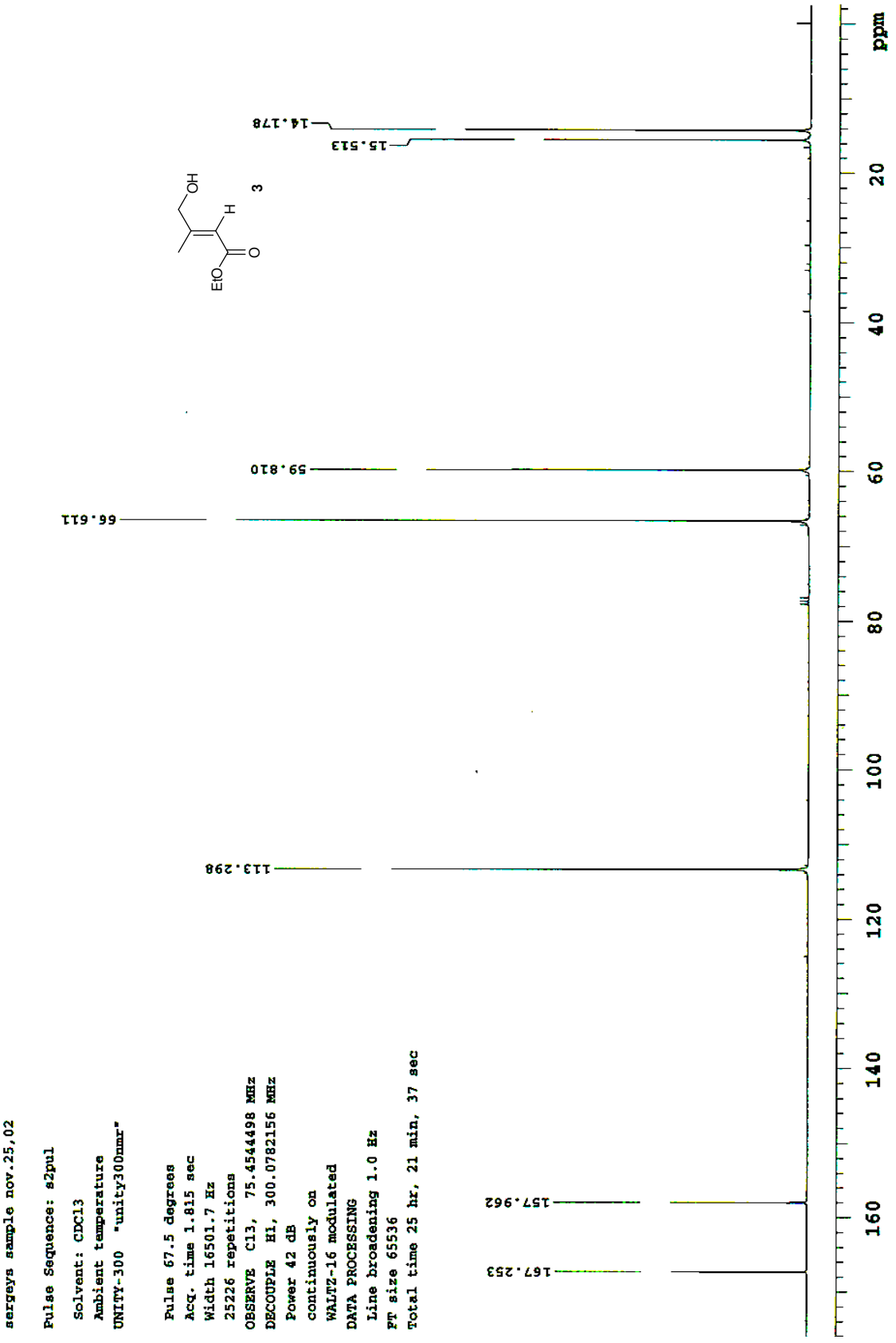
21. Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.*, **1969**, *34*, 2543-2549.

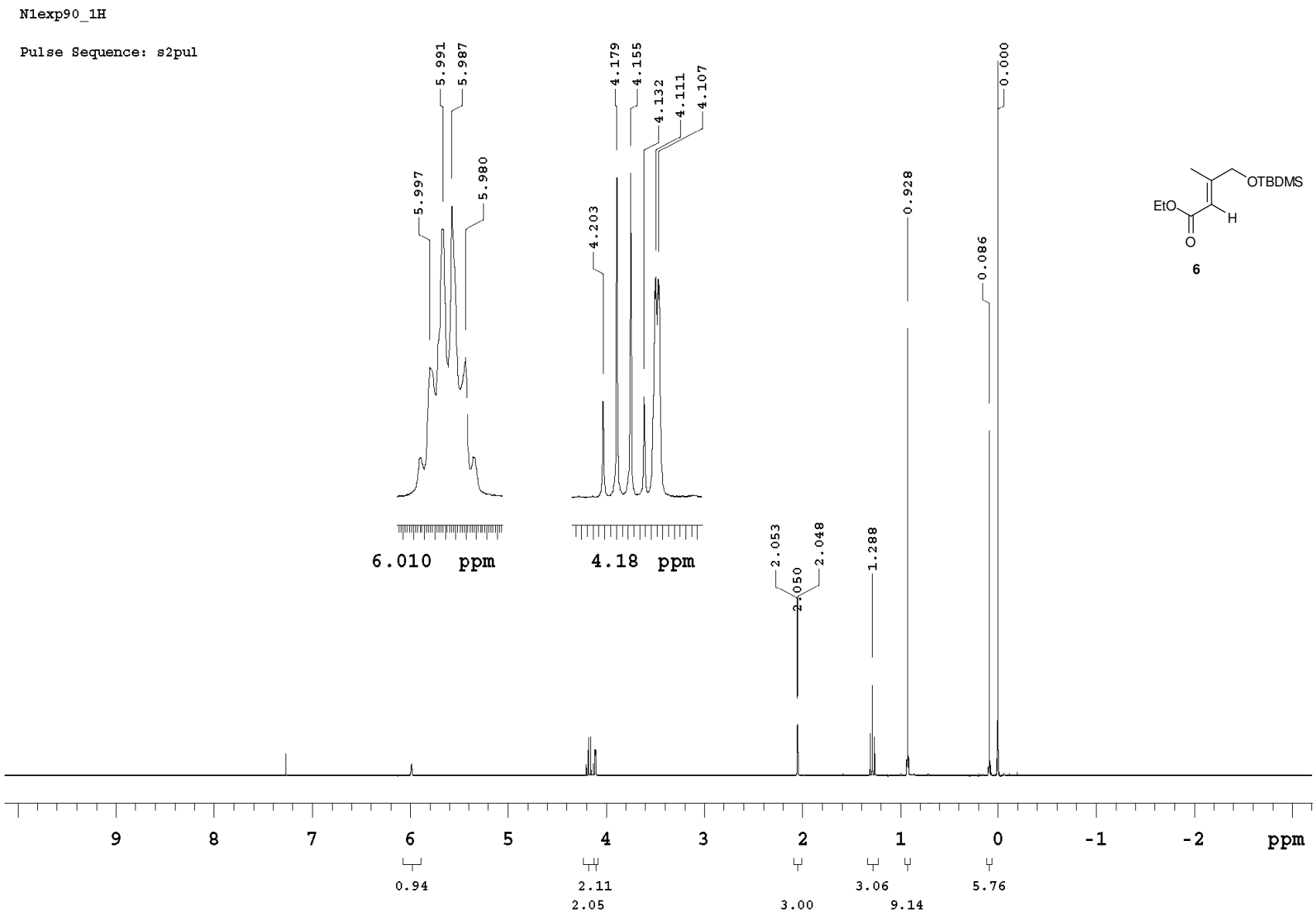
APPENDIX A

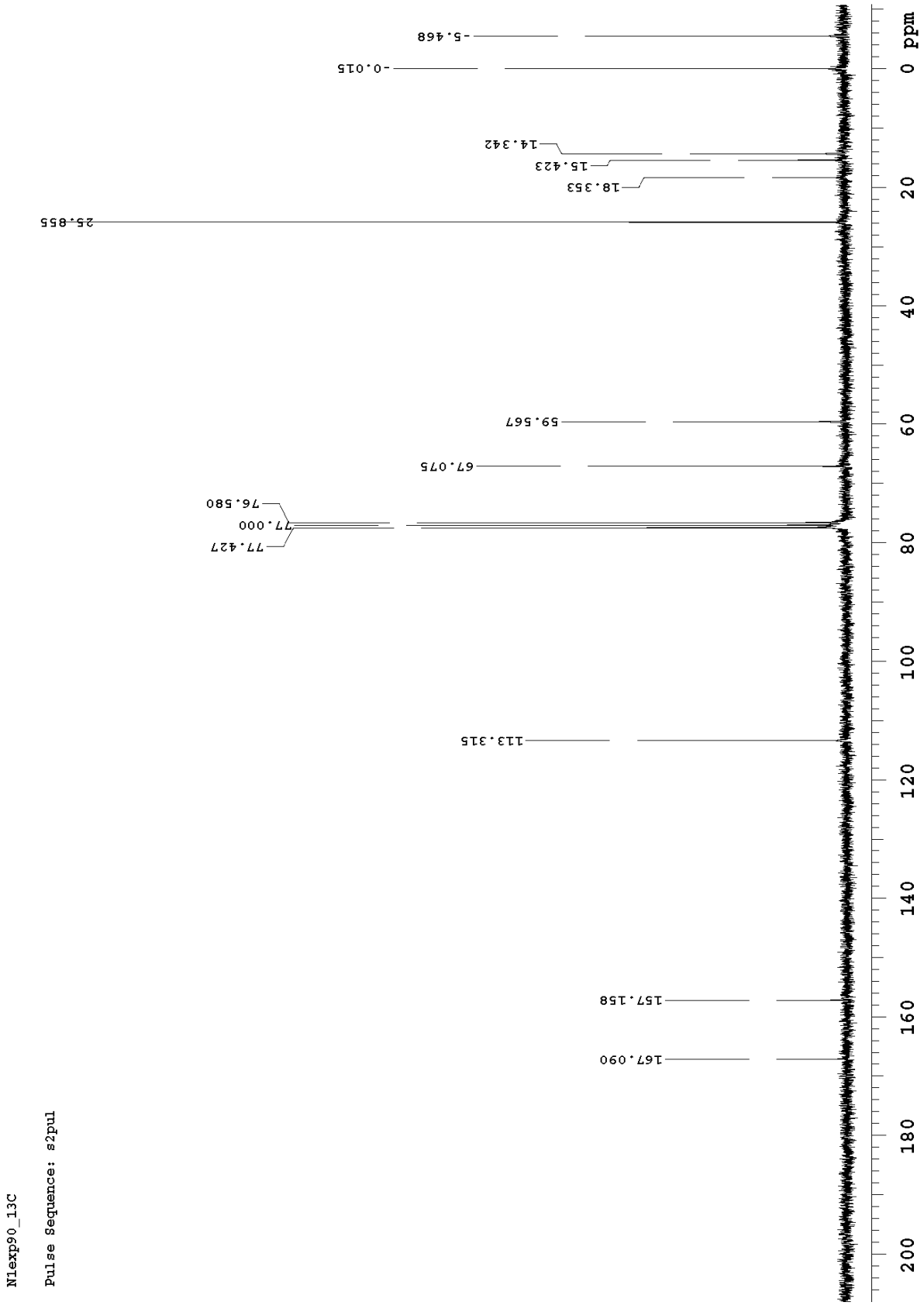
^{1}H, ^{13}C, ^{31}P NMR SPECTRA OF COMPOUNDS

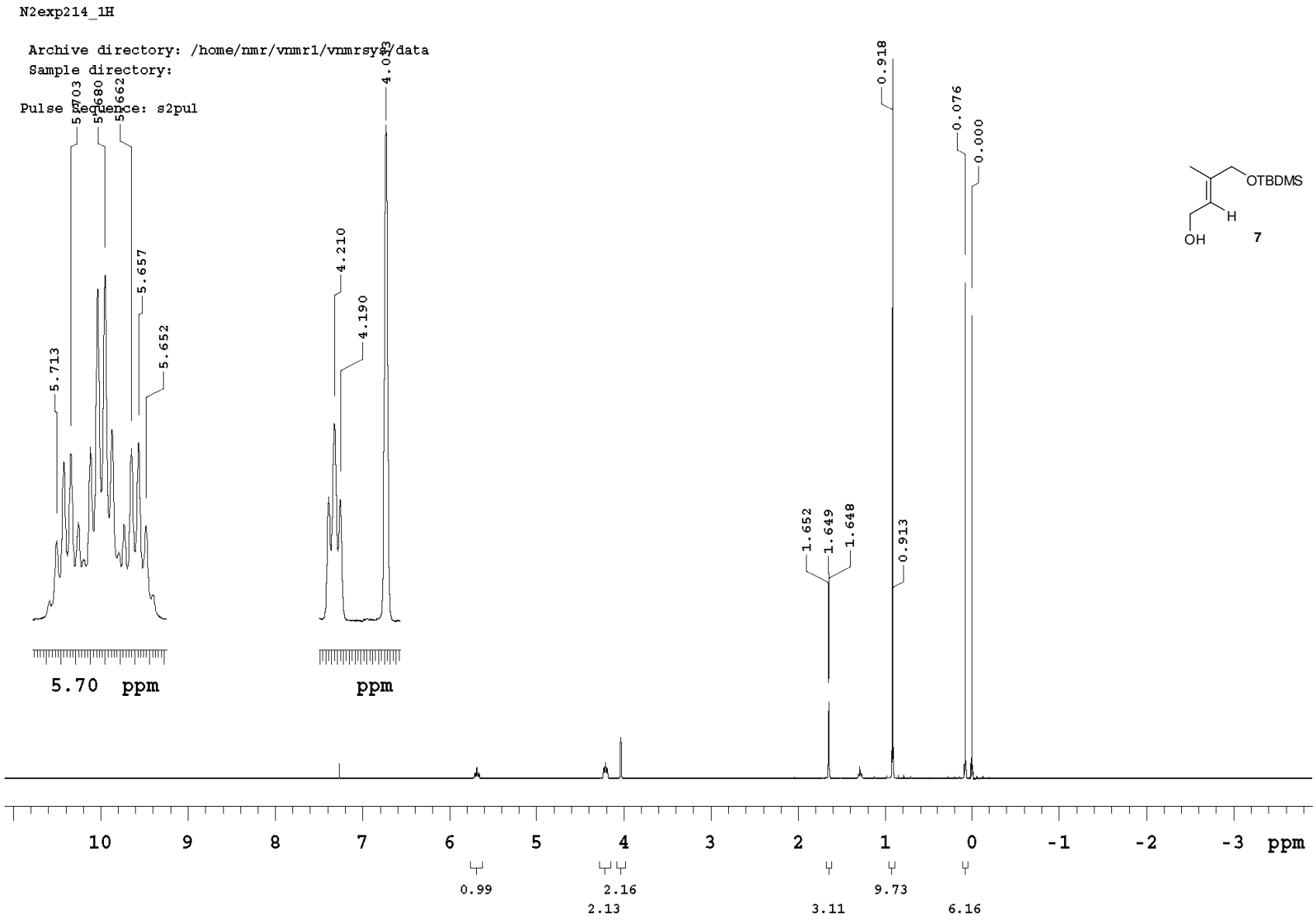
FOR CHAPTER 2

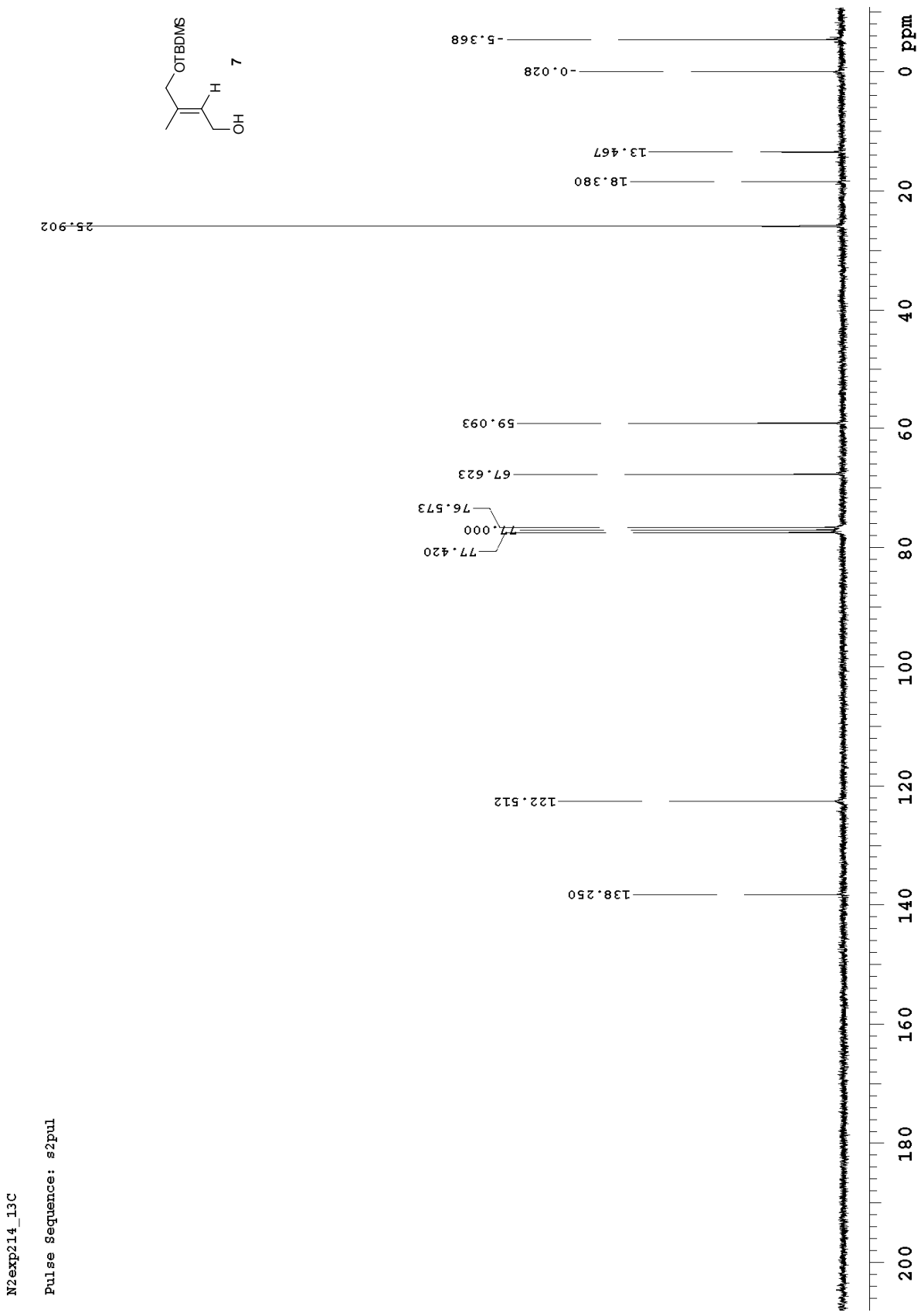

N1exp6_1H
Pulse Sequence: s2pul
5.992
5.987
5.982
5.976
4.205
4.181
4.158
4.133
2.089
2.086
2.085
1.311
1.287
1.263
0.000
1.00
4.02
0.99
3.01
2.99
ppm
OH
H
O
EtO
3

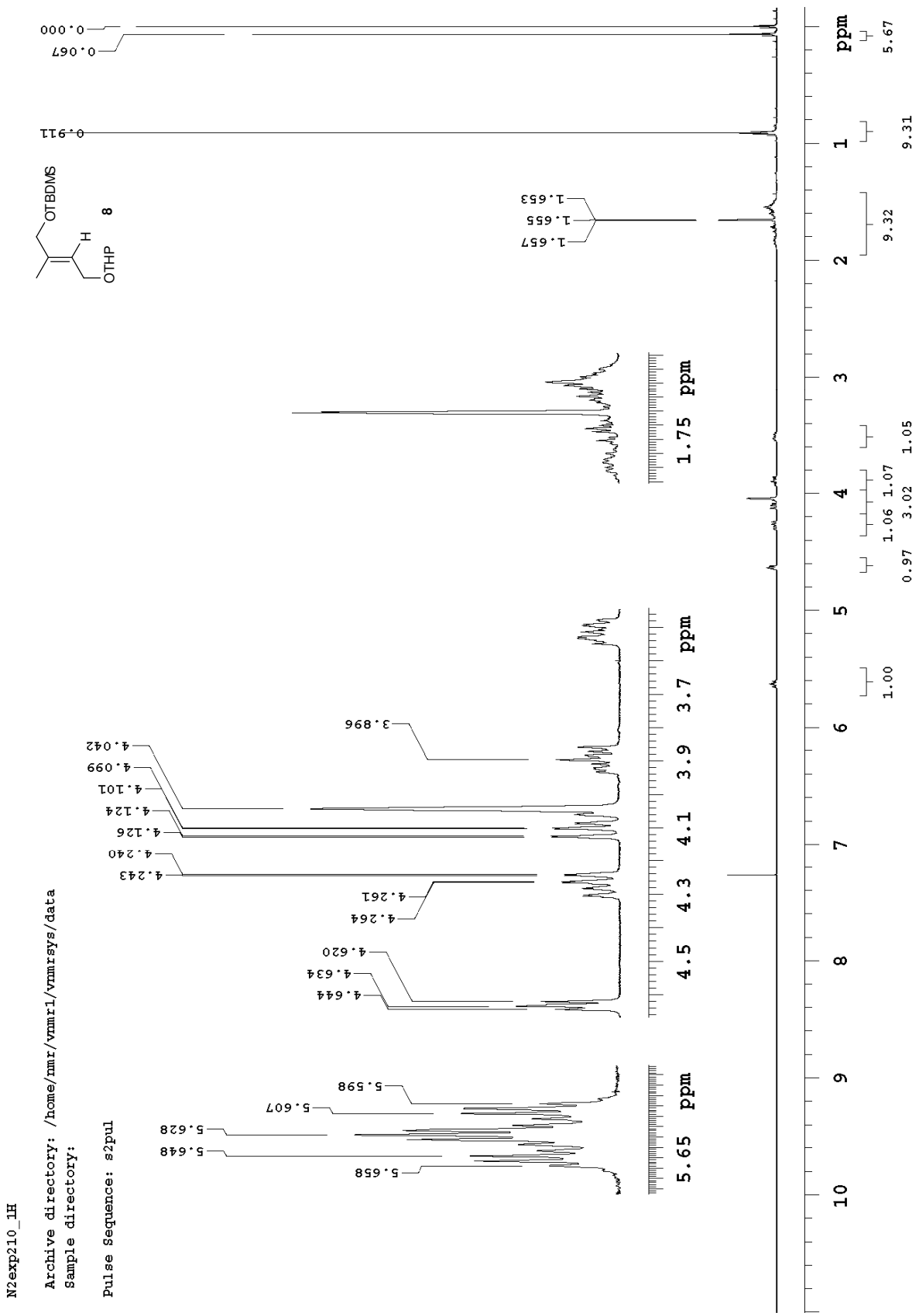

sergeys sample nov.25,02
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
UNITY-300 "unity300nmr"
Pulse 67.5 degrees
Acq. time 1.815 sec
Width 16501.7 Hz
25226 repetitions
OBSERVE C13, 75.4544498 MHz
DECOUPLE H1, 300.0782156 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 25 hr, 21 min, 37 sec
167.253
157.962
113.298
66.611
59.810
15.513
14.178
OH
H
O
EtO
3
ppm
160
140
120
100
80
60
40
20

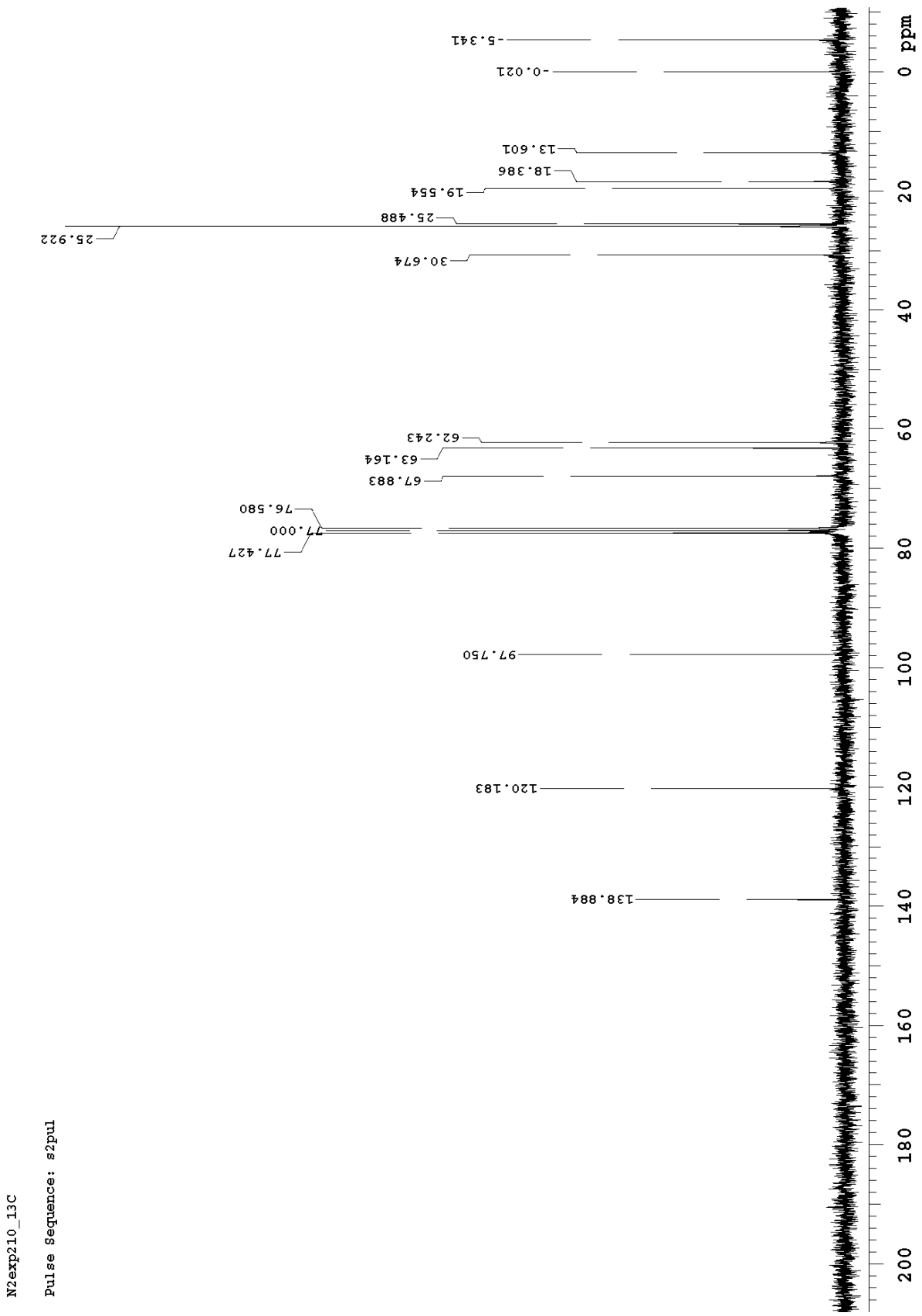

N1exp90_1H
Pulse Sequence: s2pul
5.997
5.991
5.987
5.980
4.203
4.179
4.155
4.132
4.111
4.107
6.010 ppm
4.18 ppm
2.053
2.050
2.048
1.288
0.928
0.086
0.000
OTBDMS
EtO
H
O
6
9
8
7
6
5
4
3
2
1
0
-1
-2
ppm
0.94
2.11
2.05
3.00
3.06
9.14
5.76

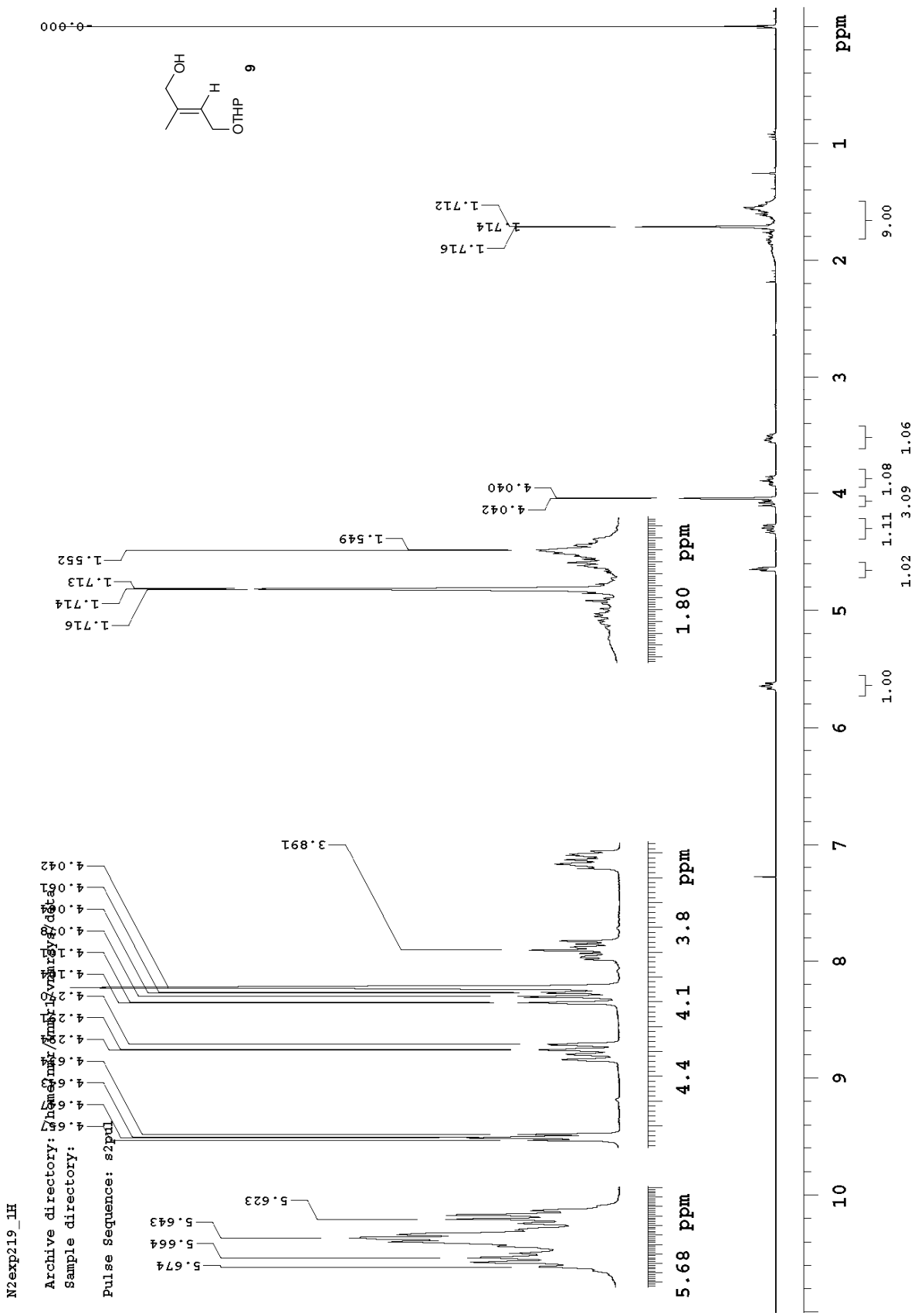

N1exp90_13C
Pulse Sequence: s2pul
167.090
157.158
113.315
77.427
77.000
76.580
67.075
59.567
25.855
18.353
15.423
14.342
-0.015
-5.468
0 ppm
20
40
60
80
100
120
140
160
180
200

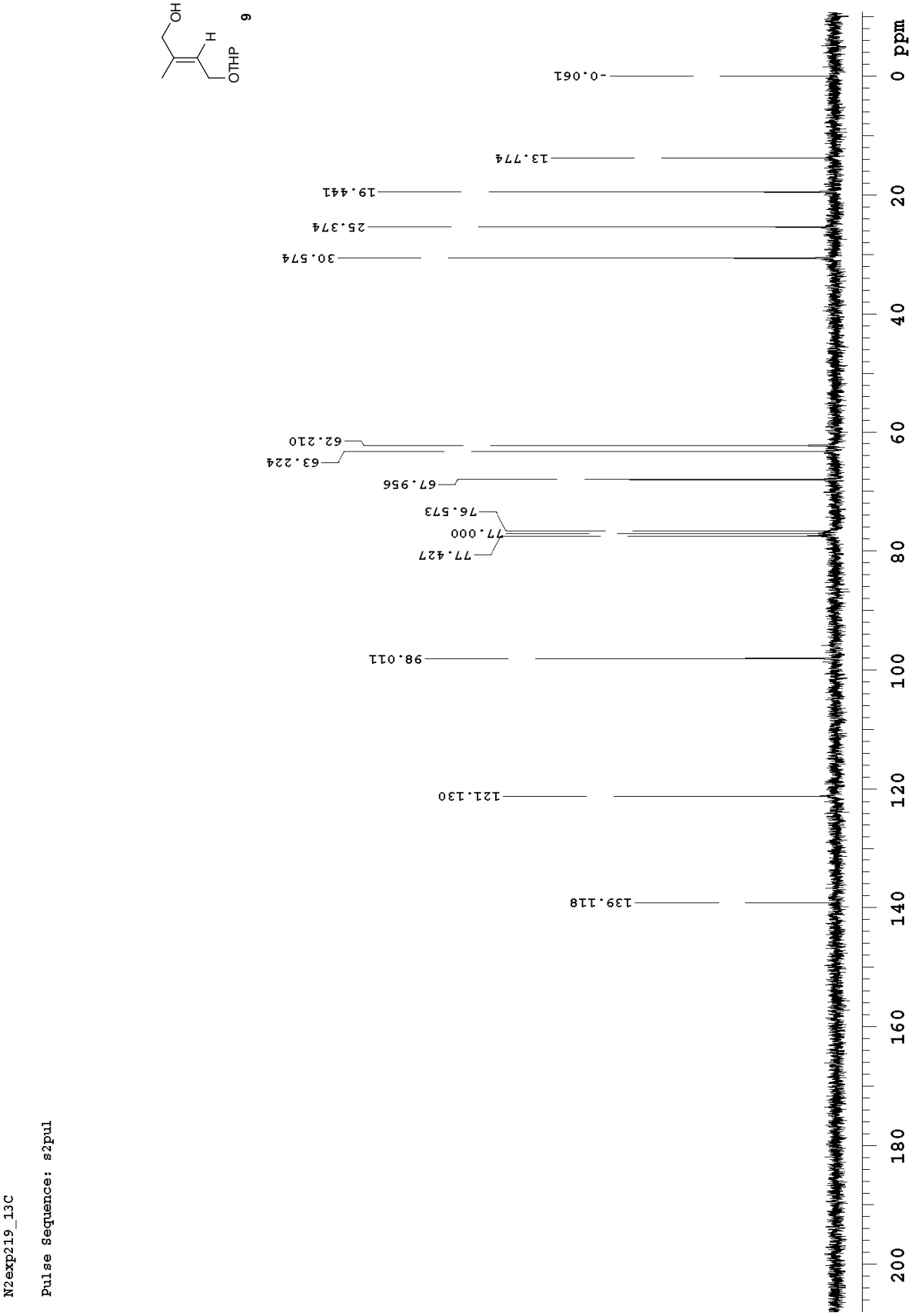

N2exp214_1H
Archive directory: /home/nmr/vnmr1/vnmrsys/data
Sample directory:
Pulse sequence: s2pul
5.703
5.680
5.662
5.657
5.652
5.713
4.210
4.190
4.083
5.70 ppm
ppm
0.918
0.076
0.000
1.652
1.649
1.648
0.913
OTBDMS
H
OH
7
10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 ppm
0.99
2.16
2.13
3.11
9.73
6.16

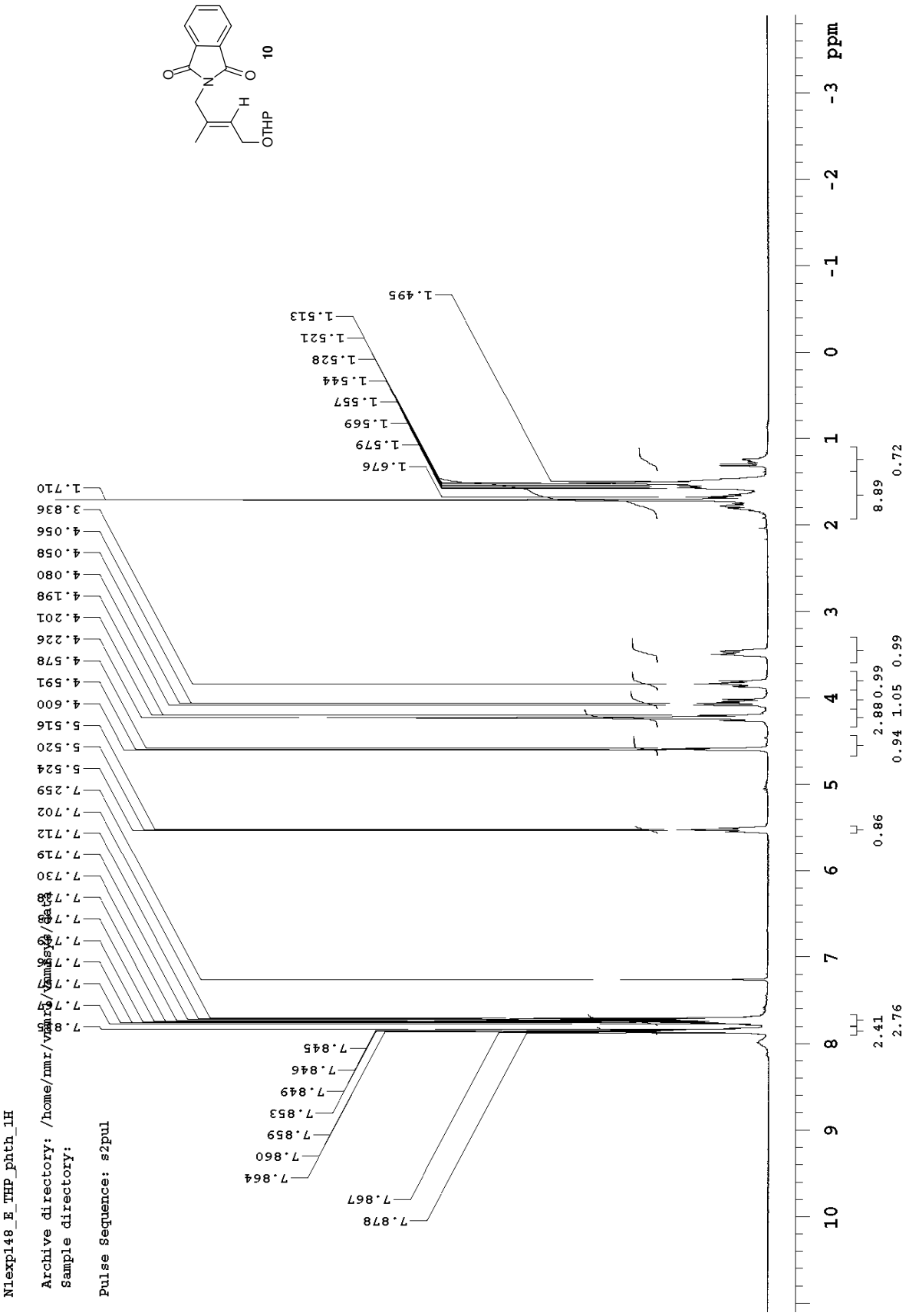

N2exp214_13C
Pulse Sequence: s2pul
OTBDMS
H
OH
7
-5.368
-0.028
13.467
18.380
25.902
59.093
67.623
76.573
77.000
77.420
122.512
138.250
0 ppm
20
40
60
80
100
120
140
160
180
200

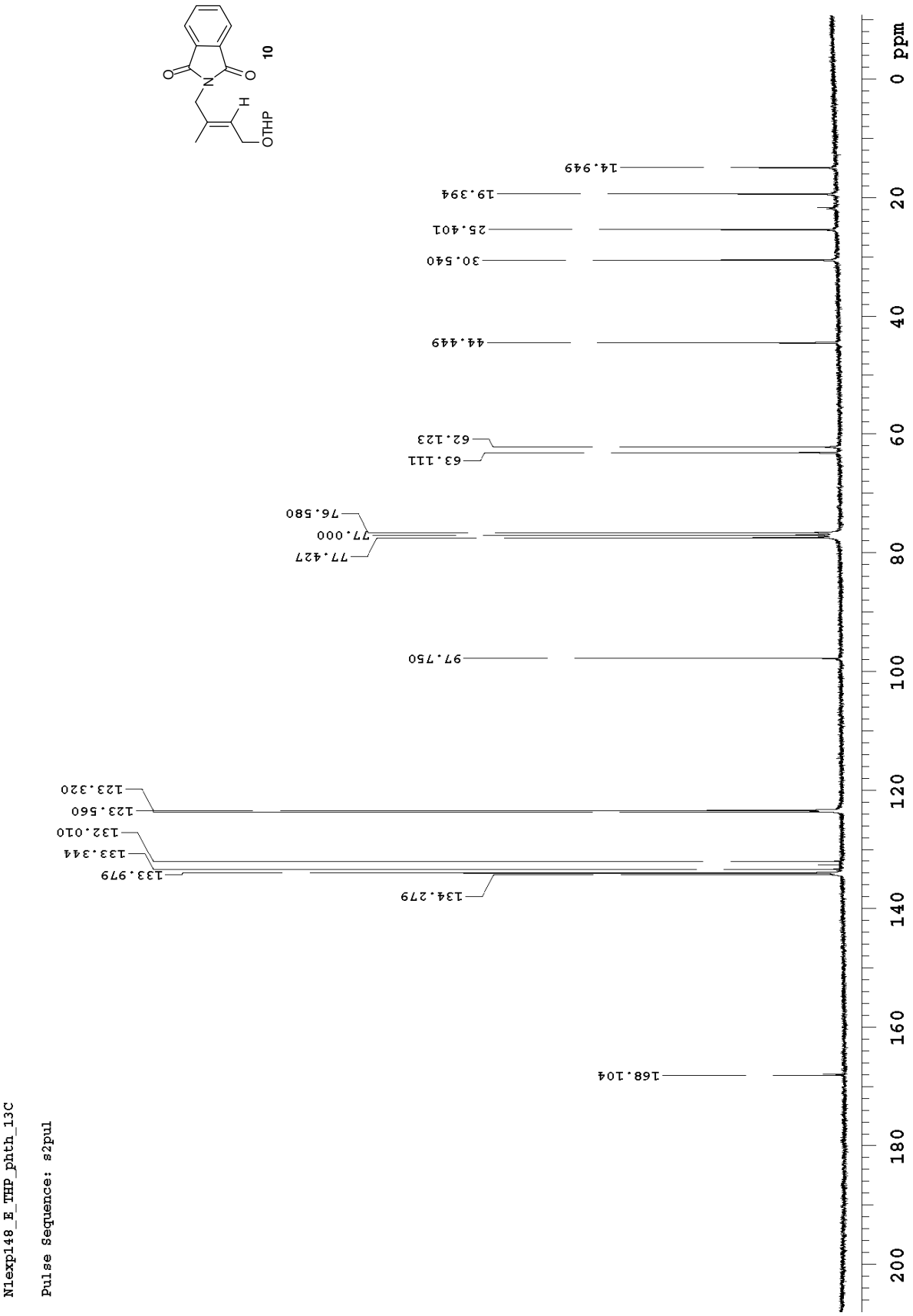

N2exp210_1H
Archive directory: /home/nmr/vnmr1/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
OTBDMS
H
OTHP
8
0.000
0.067
0.911
1.653
1.655
1.657
5.598
5.607
5.628
5.648
5.658
4.620
4.634
4.644
4.243
4.240
4.126
4.124
4.101
4.099
4.042
3.896
4.261
4.264
5.65 ppm
4.5 4.3 4.1 3.9 3.7 ppm
1.75 ppm
ppm
1.00
0.97 1.06 1.07
3.02 1.05
9.32
9.31
5.67

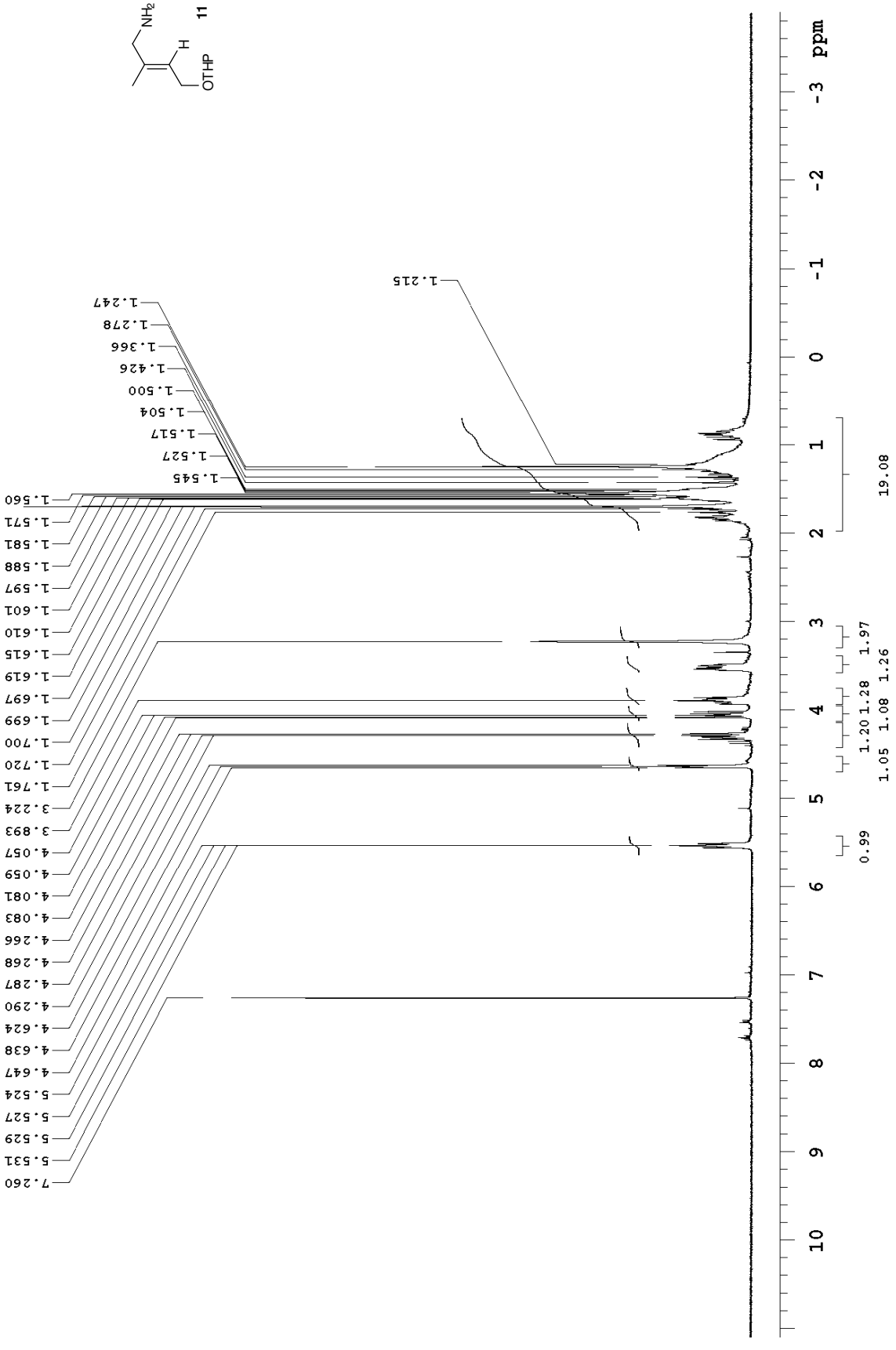

N2exp210_13C
Pulse Sequence: s2pul
138.884
120.183
97.750
77.427
77.000
76.580
67.883
63.164
62.243
30.674
25.922
25.488
19.554
18.386
13.601
-0.021
-5.341
0 ppm
20
40
60
80
100
120
140
160
180
200

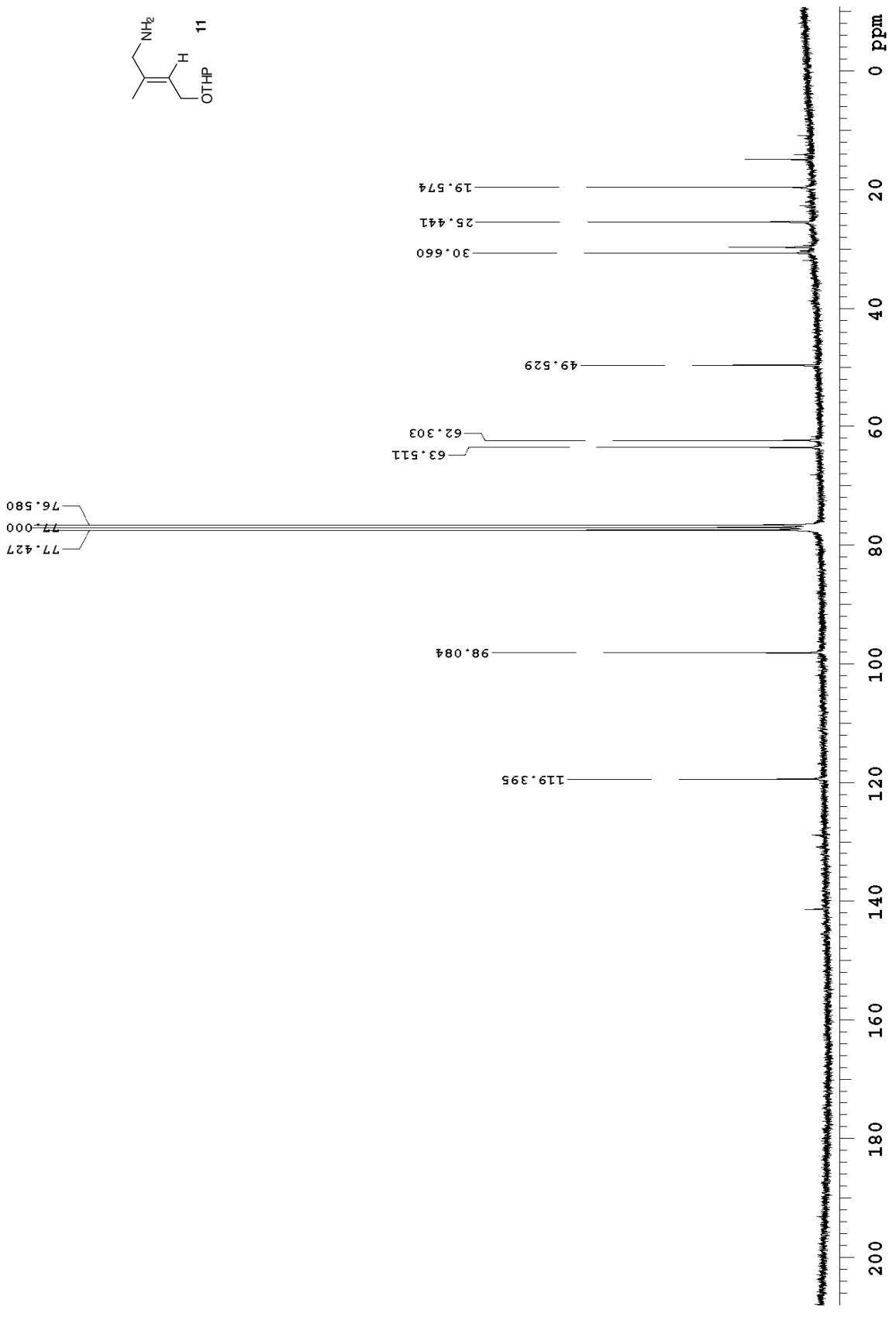

N2exp219_1H
Archive directory: /home/nmr/data
Sample directory:
Pulse Sequence: s2pul
5.674
5.664
5.643
5.623
4.657
4.647
4.633
4.624
4.242
4.232
4.222
4.212
4.161
4.151
4.141
4.131
4.080
4.070
4.061
4.042
3.891
1.716
1.714
1.713
1.552
1.549
4.042
4.040
1.716
1.714
1.712
OH
H
OTHP
9
ppm
5.68 ppm
4.4 4.1 3.8 ppm
1.80 ppm
1.00
1.02
1.11
1.08
3.09
1.06
9.00

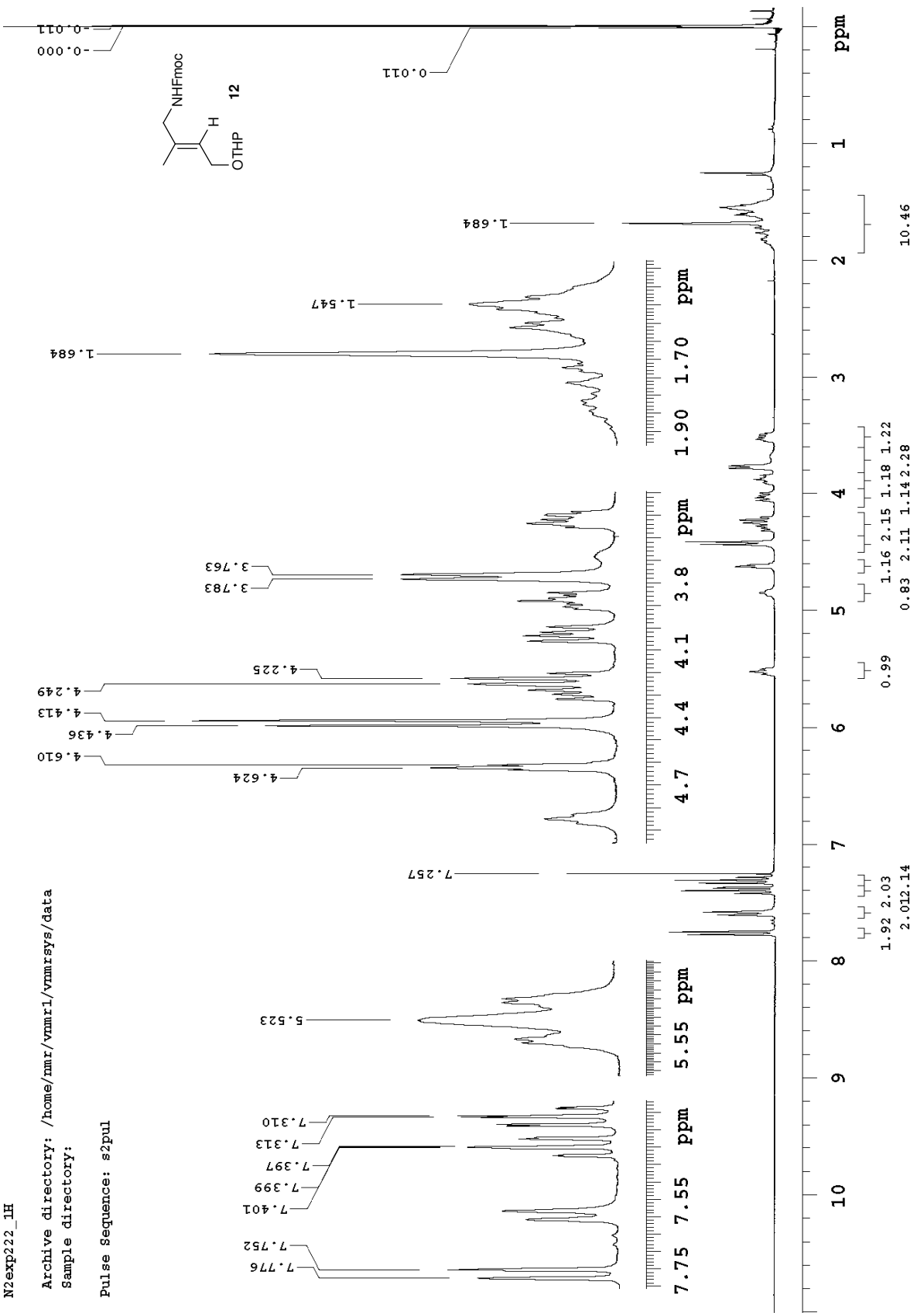

N2exp219_13C
Pulse Sequence: s2pul
OH
H
OTHP
9
-0.061
13.774
19.441
25.374
30.574
62.210
63.224
67.956
76.573
77.000
77.427
98.011
121.130
139.118
0 ppm
20
40
60
80
100
120
140
160
180
200

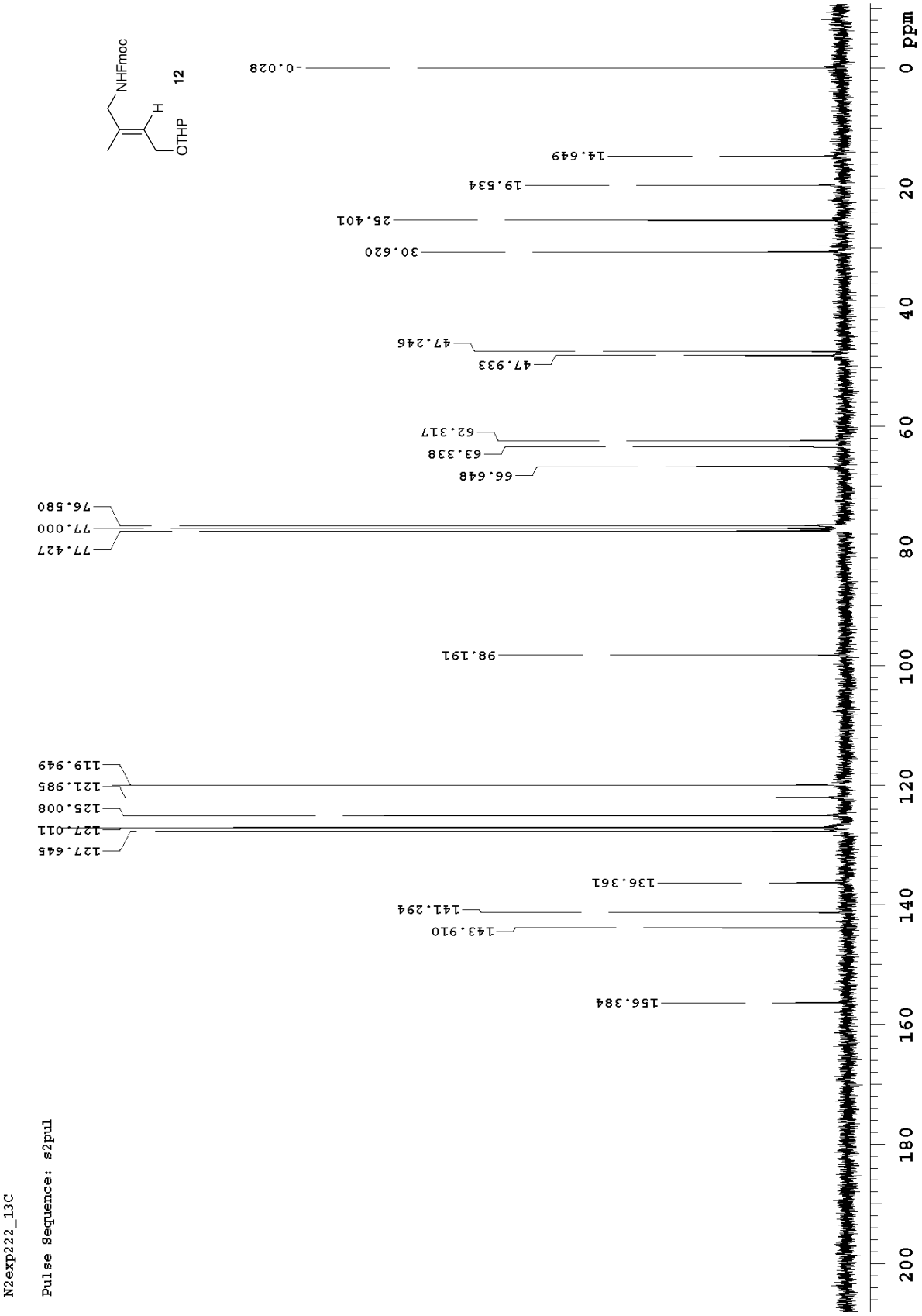

10
OTHP
H
N
O
O
N1exp148_E_THP_phth_1H
Archive directory: /home/nmr/vnmrs/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
ppm
1.710
3.836
4.056
4.058
4.080
4.198
4.201
4.226
4.578
4.591
4.600
5.516
5.520
5.524
7.259
7.702
7.712
7.719
7.730
7.845
7.846
7.849
7.853
7.859
7.860
7.864
7.867
7.878
1.495
1.513
1.521
1.528
1.544
1.557
1.569
1.579
1.676
2.41
2.76
0.86
2.88
0.99
0.94
1.05
0.99
8.89
0.72

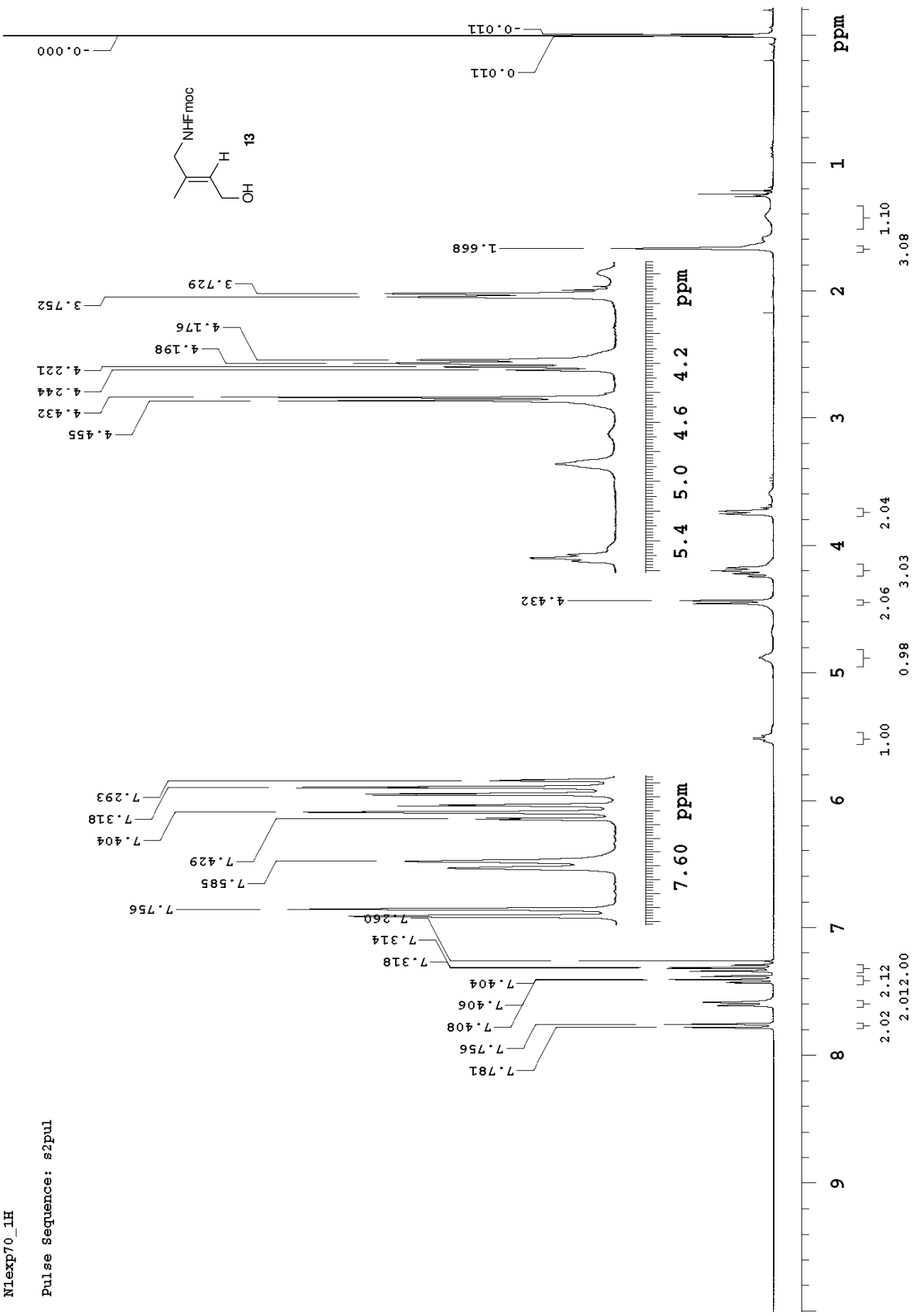

N1exp148_E_THP_phth_13C
Pulse Sequence: s2pul
10
OTHP
H
N
O
O
168.104
134.279
133.979
133.344
132.010
123.560
123.320
97.750
77.427
77.000
76.580
63.111
62.123
44.449
30.540
25.401
19.394
14.949
0 ppm
20
40
60
80
100
120
140
160
180
200

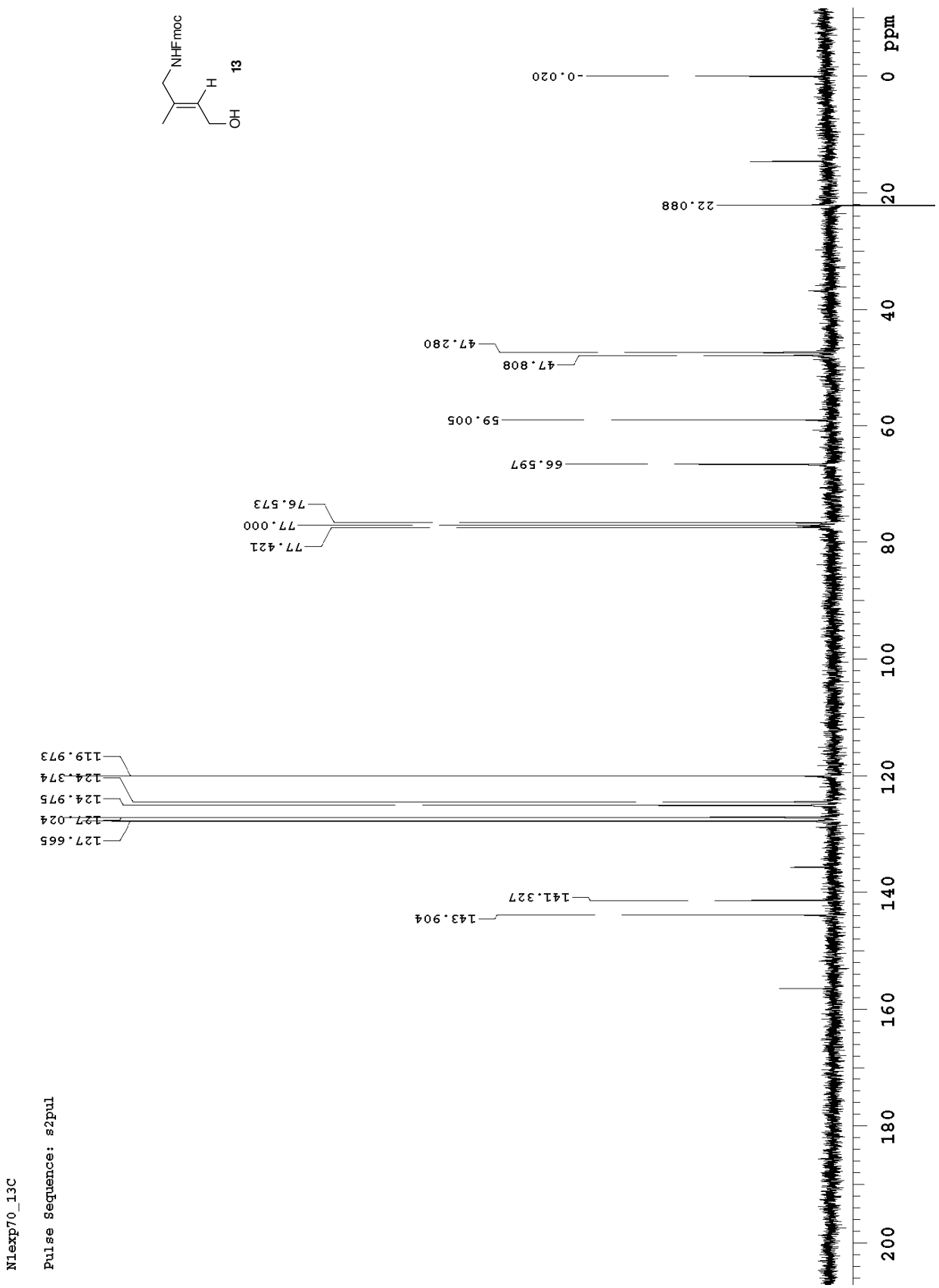

11
NH2
H
OTHP
ppm
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
0.99
1.05
1.20
1.28
1.08
1.97
1.26
19.08
7.260
5.531
5.529
5.527
5.524
4.647
4.638
4.624
4.290
4.287
4.268
4.266
4.083
4.081
4.059
4.057
3.893
3.224
1.761
1.720
1.700
1.699
1.697
1.619
1.615
1.610
1.601
1.597
1.588
1.581
1.571
1.560
1.545
1.527
1.517
1.504
1.500
1.426
1.366
1.278
1.247
1.215

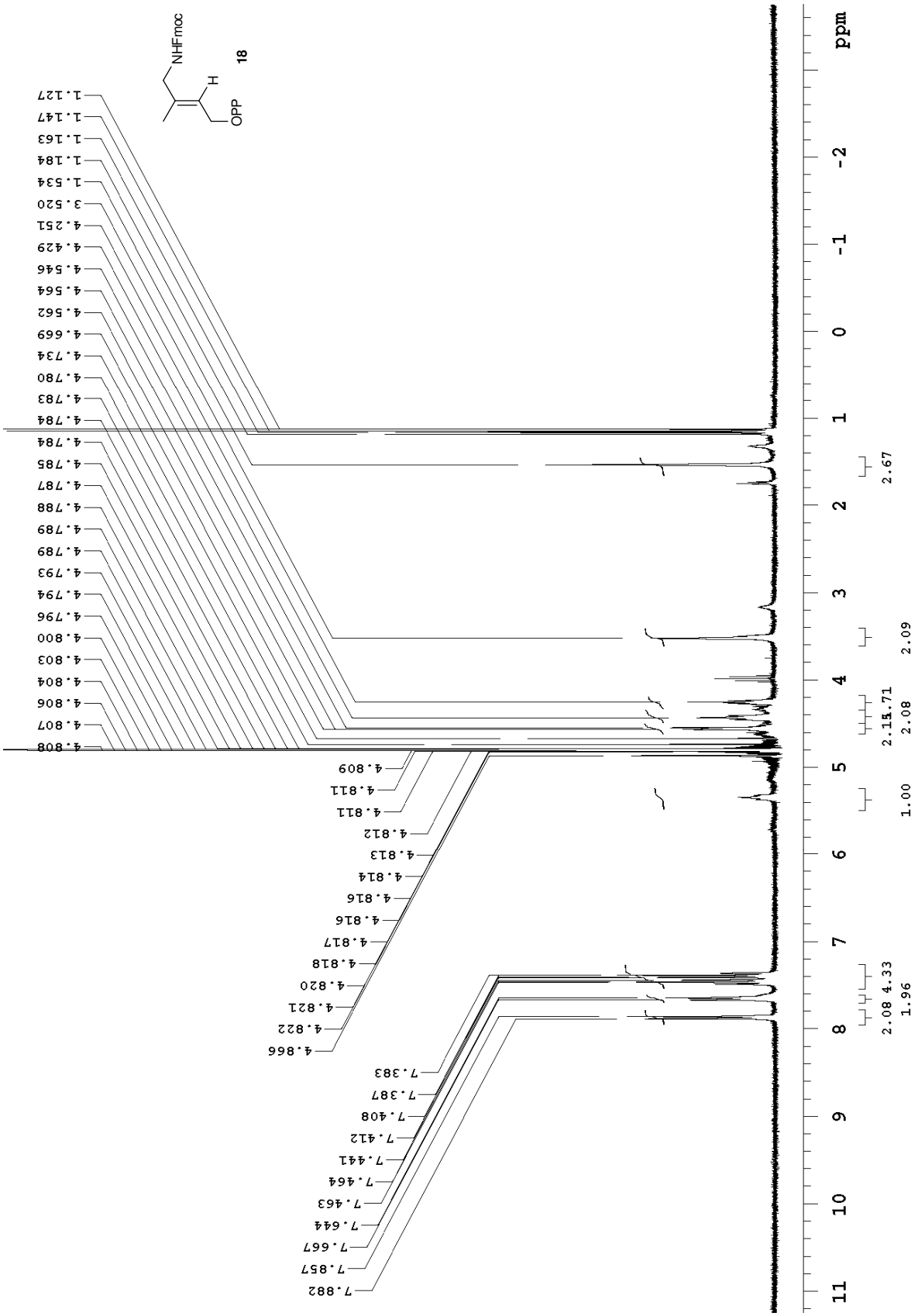

11
NH2
H
OTHP
0 ppm
20
40
60
80
100
120
140
160
180
200
19.574
25.441
30.660
49.529
62.303
63.511
76.580
77.000
77.427
98.084
119.395

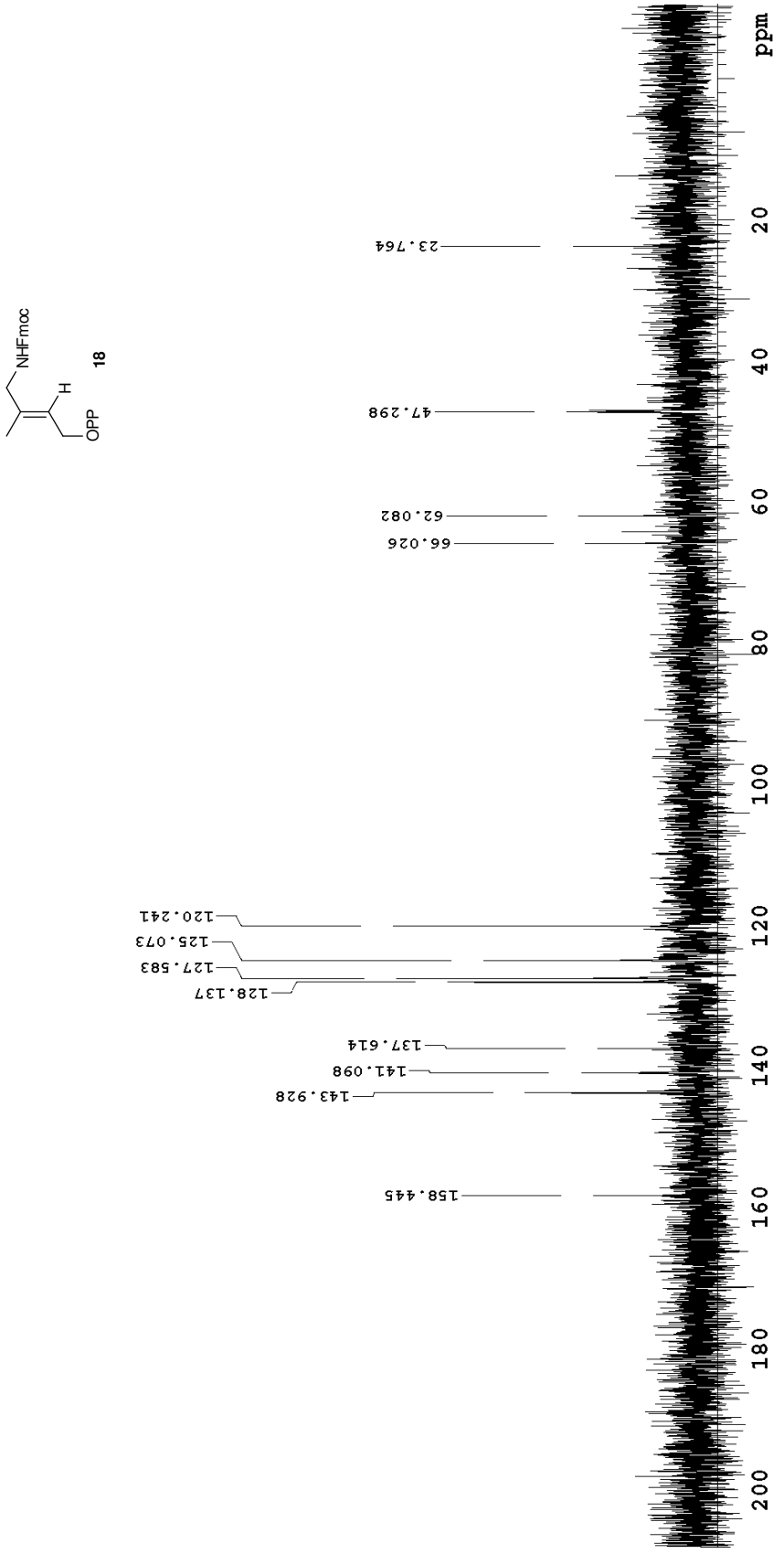

N2exp222_1H
Archive directory: /home/nmr/vnmr1/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
NHFmoc
H
OTHP
12
-0.011
-0.000
0.011
1.684
1.547
1.684
3.763
3.783
4.225
4.249
4.413
4.436
4.610
4.624
7.257
5.523
7.310
7.313
7.397
7.399
7.401
7.752
7.776
ppm
10.46
0.99
1.92 2.03
2.012.14
1.16 2.15 1.18 1.22
0.83 2.11 1.142.28

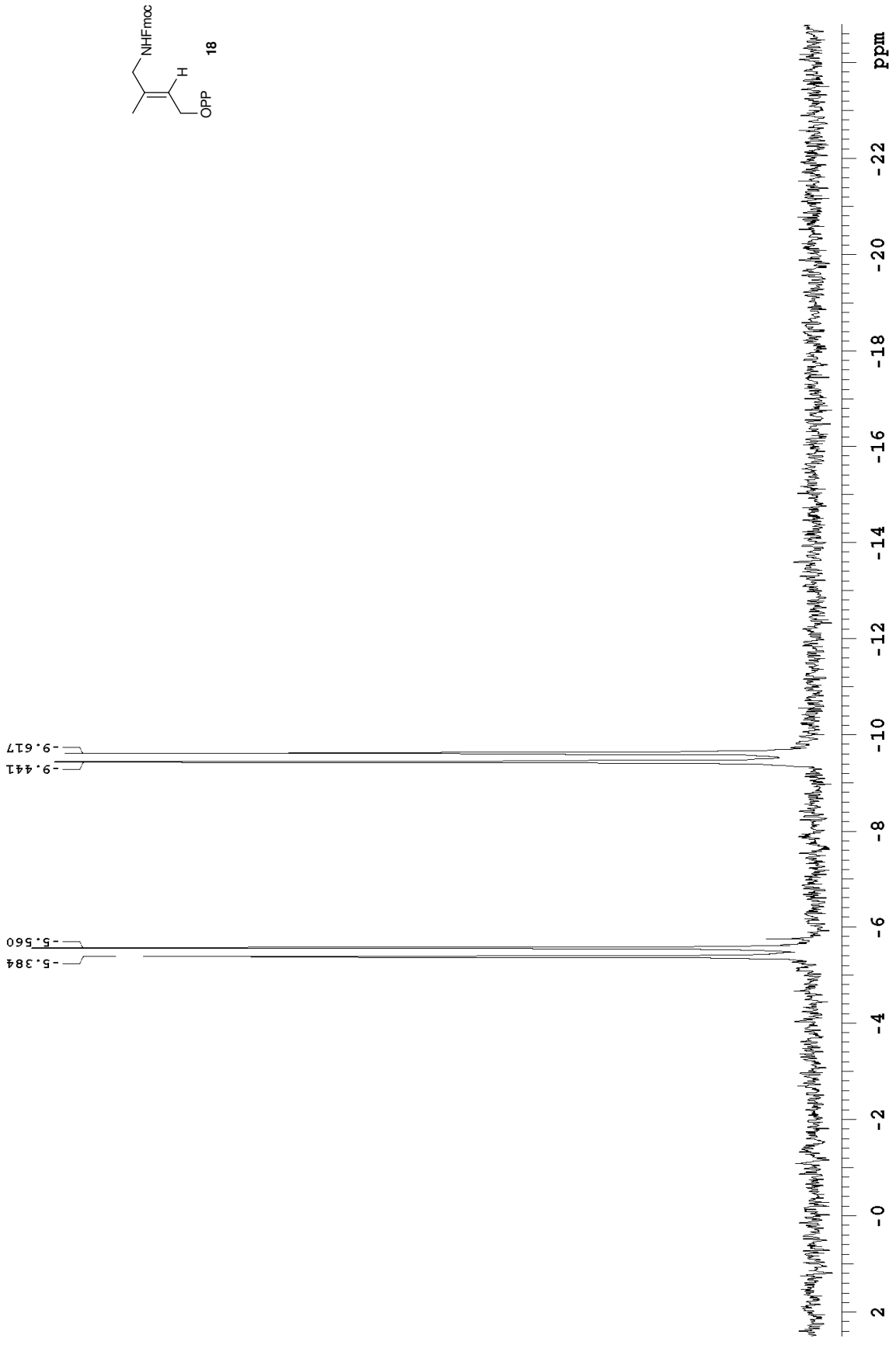

N2exp222_13C
Pulse Sequence: s2pul
NHFmoc
H
OTHP
12
-0.028
14.649
19.534
25.401
30.620
47.246
47.933
62.317
63.338
66.648
76.580
77.000
77.427
98.191
119.949
121.985
125.008
127.011
127.645
136.361
141.294
143.910
156.384
0 ppm
20
40
60
80
100
120
140
160
180
200

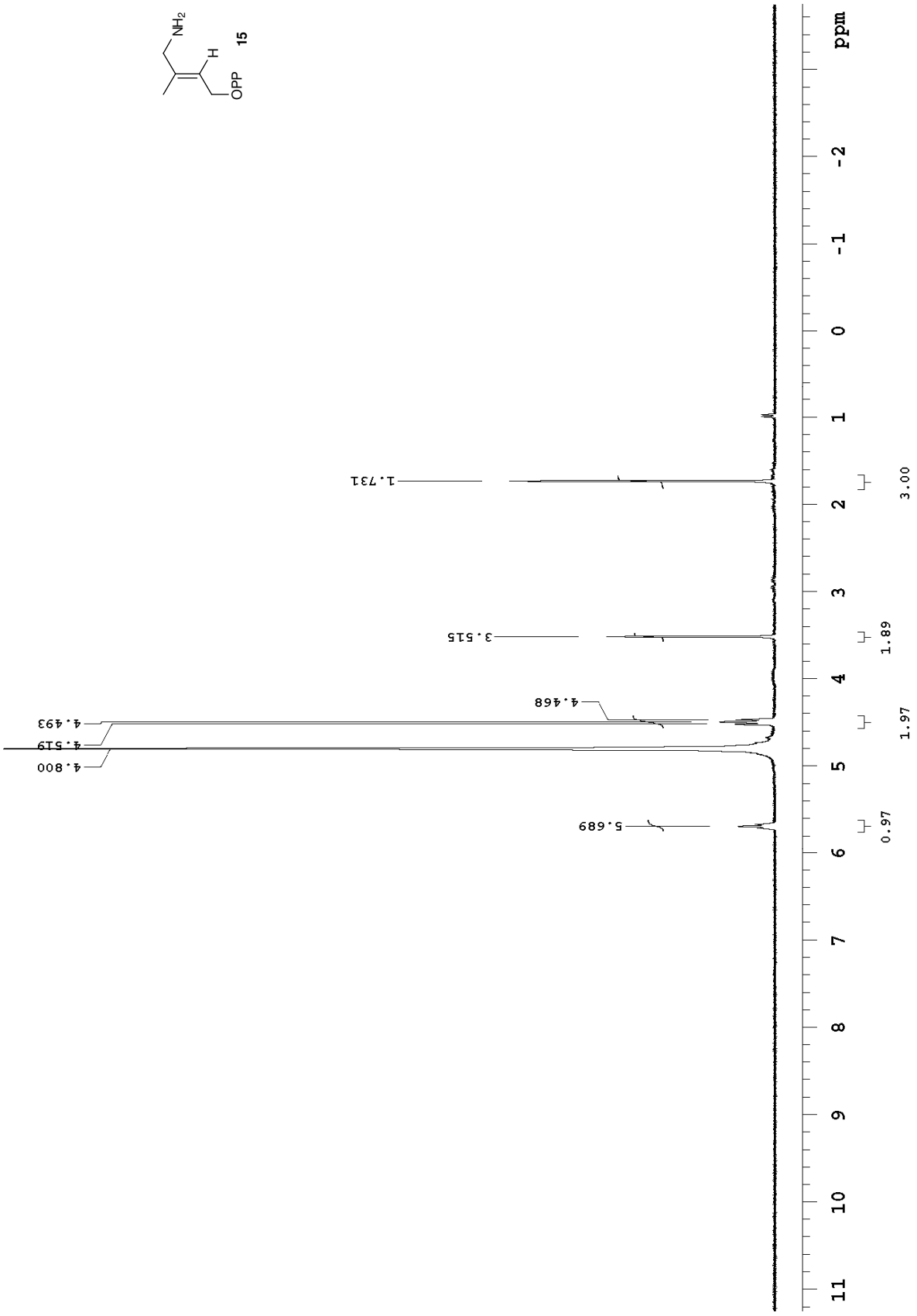

N1exp70_1H
Pulse Sequence: s2pul
NHFmoc
OH
13
-0.000
0.011
-0.011
1.668
3.729
3.752
4.176
4.198
4.221
4.244
4.432
4.455
4.432
7.293
7.318
7.404
7.429
7.585
7.756
7.260
7.314
7.318
7.404
7.406
7.408
7.756
7.781
7.60 ppm
5.4 5.0 4.6 4.2 ppm
ppm
1.00
0.98
2.06
3.03
2.04
1.10
3.08
2.02 2.12
2.012.00

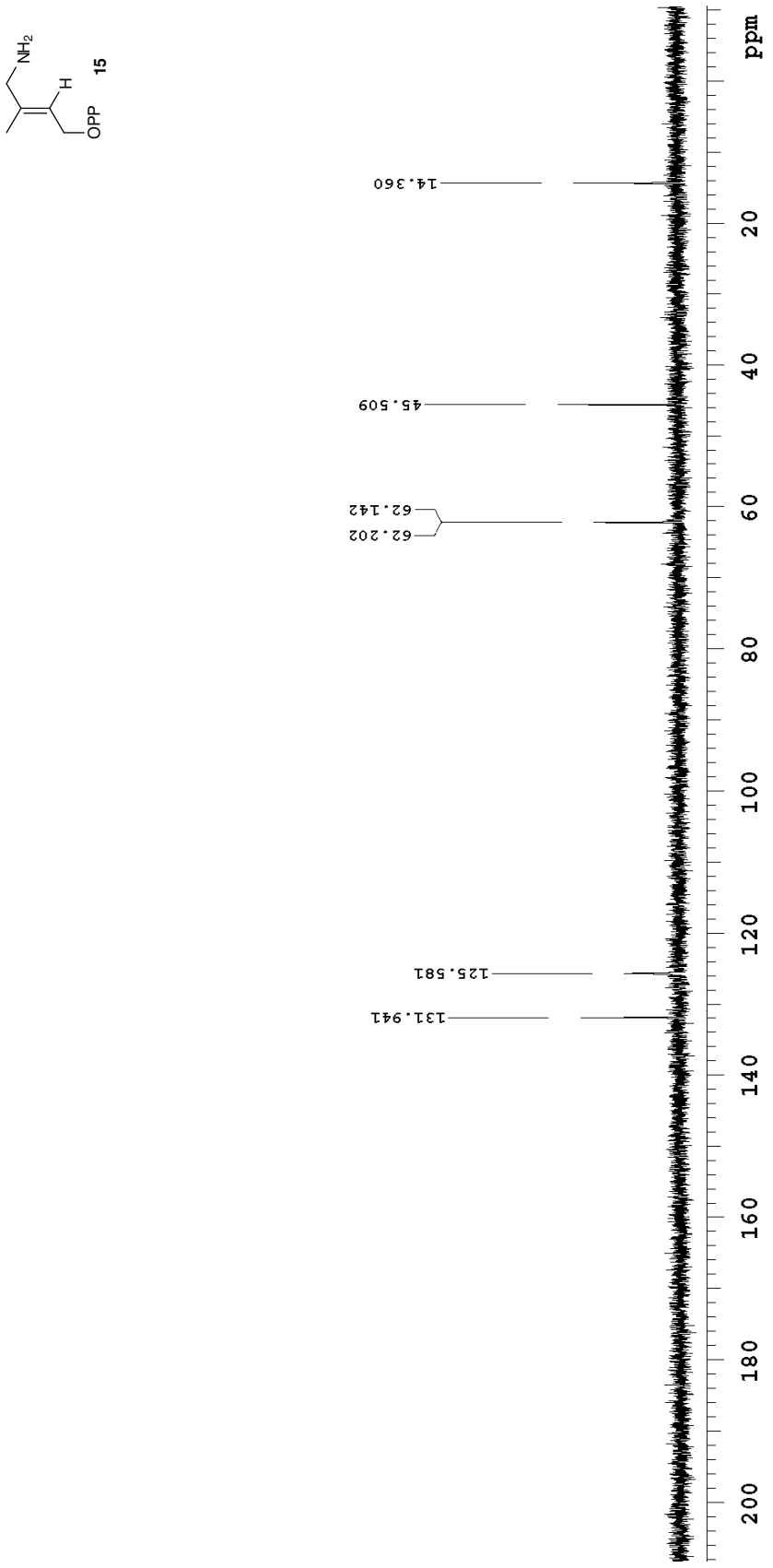

NHFmoc
H
OH
13
-0.020
22.088
47.280
47.808
59.005
66.597
76.573
77.000
77.421
119.973
124.374
124.975
127.024
127.665
141.327
143.904
ppm
0
20
40
60
80
100
120
140
160
180
200
Pulse Sequence: s2pul
N1exp70_13C

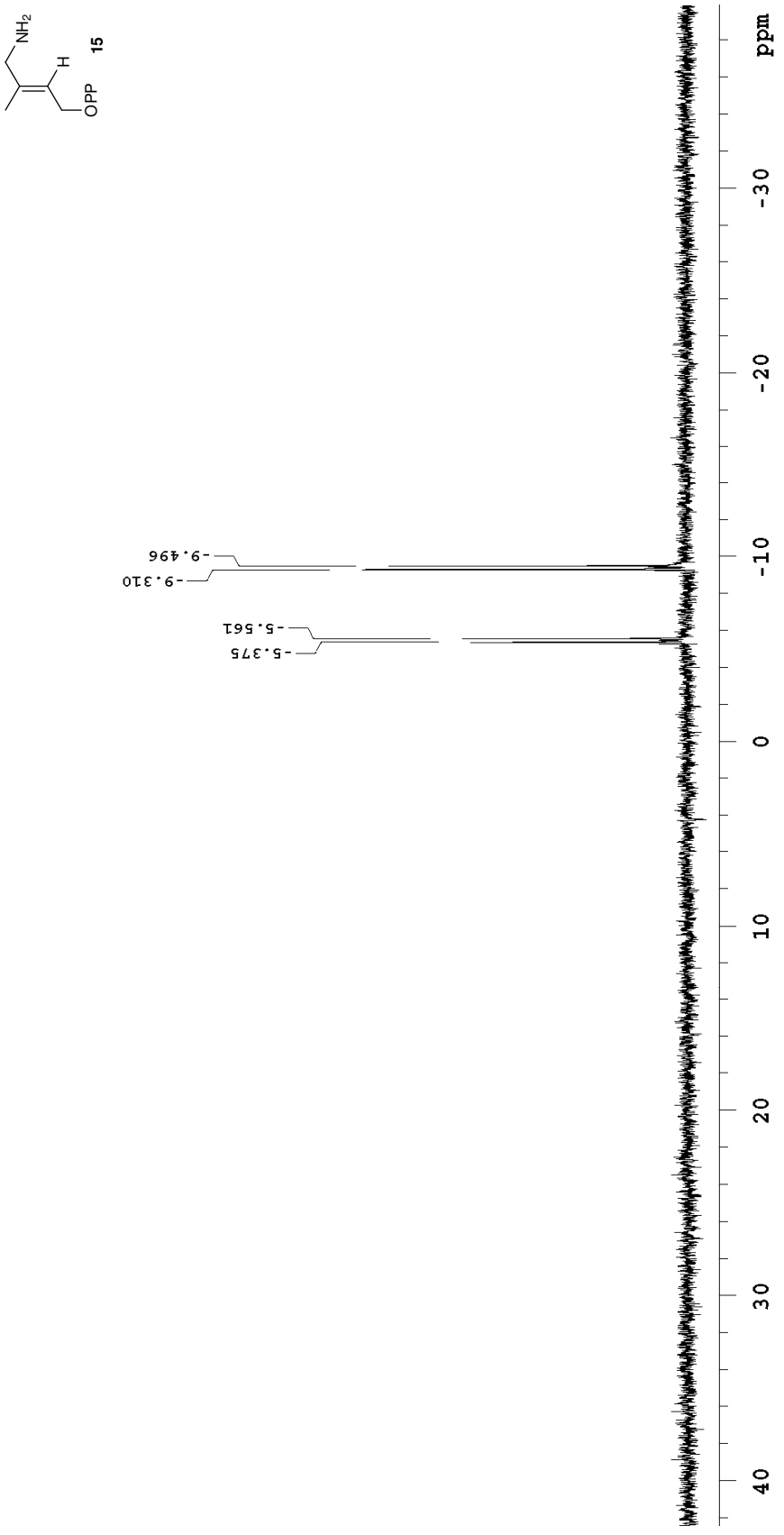

NHFmoc
H
OPP
18
1.127
1.147
1.163
1.184
1.534
3.520
4.251
4.429
4.546
4.564
4.562
4.669
4.734
4.780
4.783
4.784
4.784
4.785
4.787
4.788
4.789
4.789
4.793
4.794
4.796
4.800
4.803
4.804
4.806
4.807
4.808
4.809
4.811
4.811
4.812
4.813
4.814
4.816
4.816
4.817
4.818
4.820
4.821
4.822
4.866
7.383
7.387
7.408
7.412
7.441
7.464
7.463
7.644
7.667
7.857
7.882
ppm
2.67
2.11
1.71
2.08
2.09
1.00
2.08
4.33
1.96

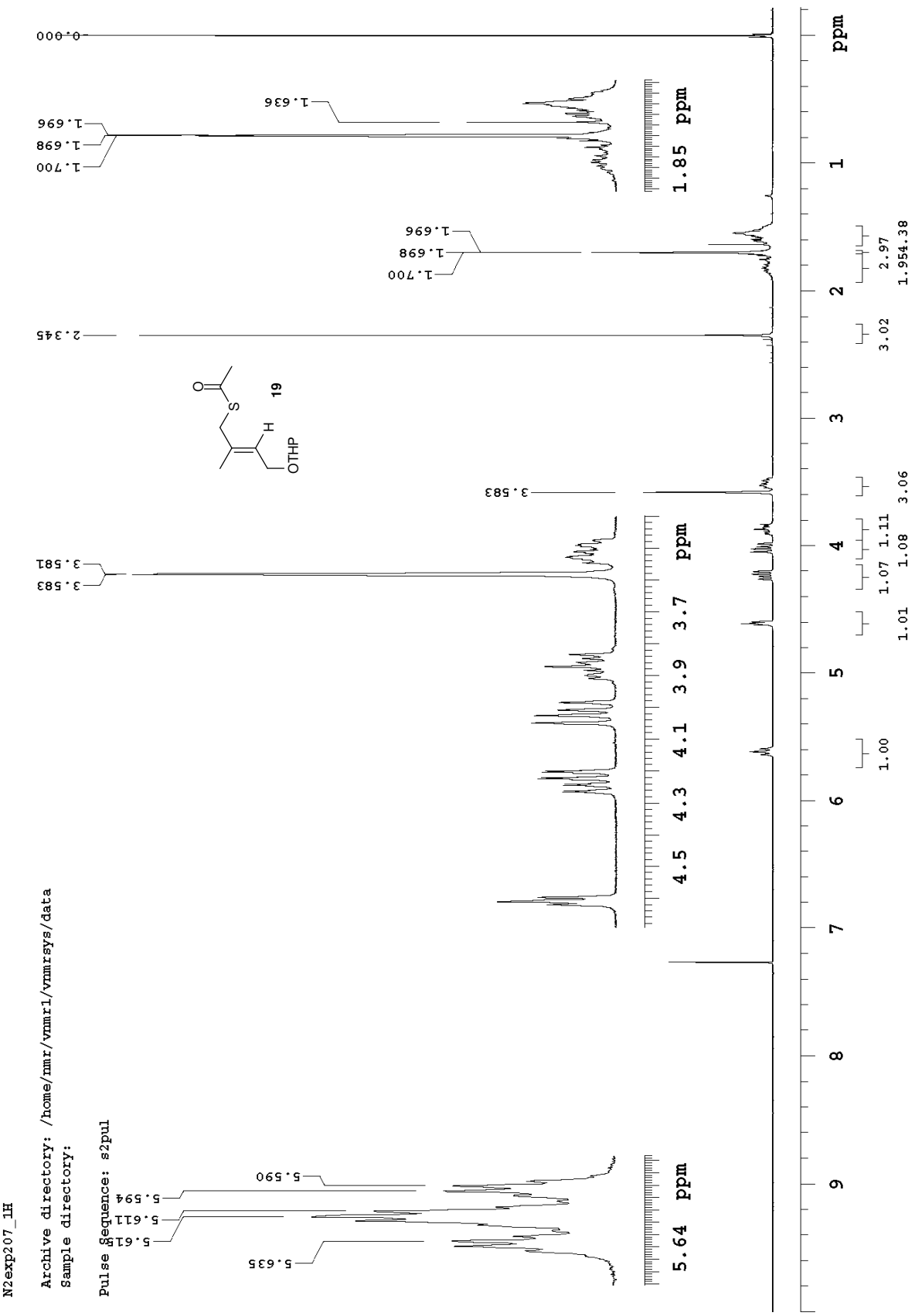

NHFmoc
H
OPP
18
158.445
143.928
141.098
137.614
128.137
127.583
125.073
120.241
66.026
62.082
47.298
23.764
ppm
200
180
160
140
120
100
80
60
40
20

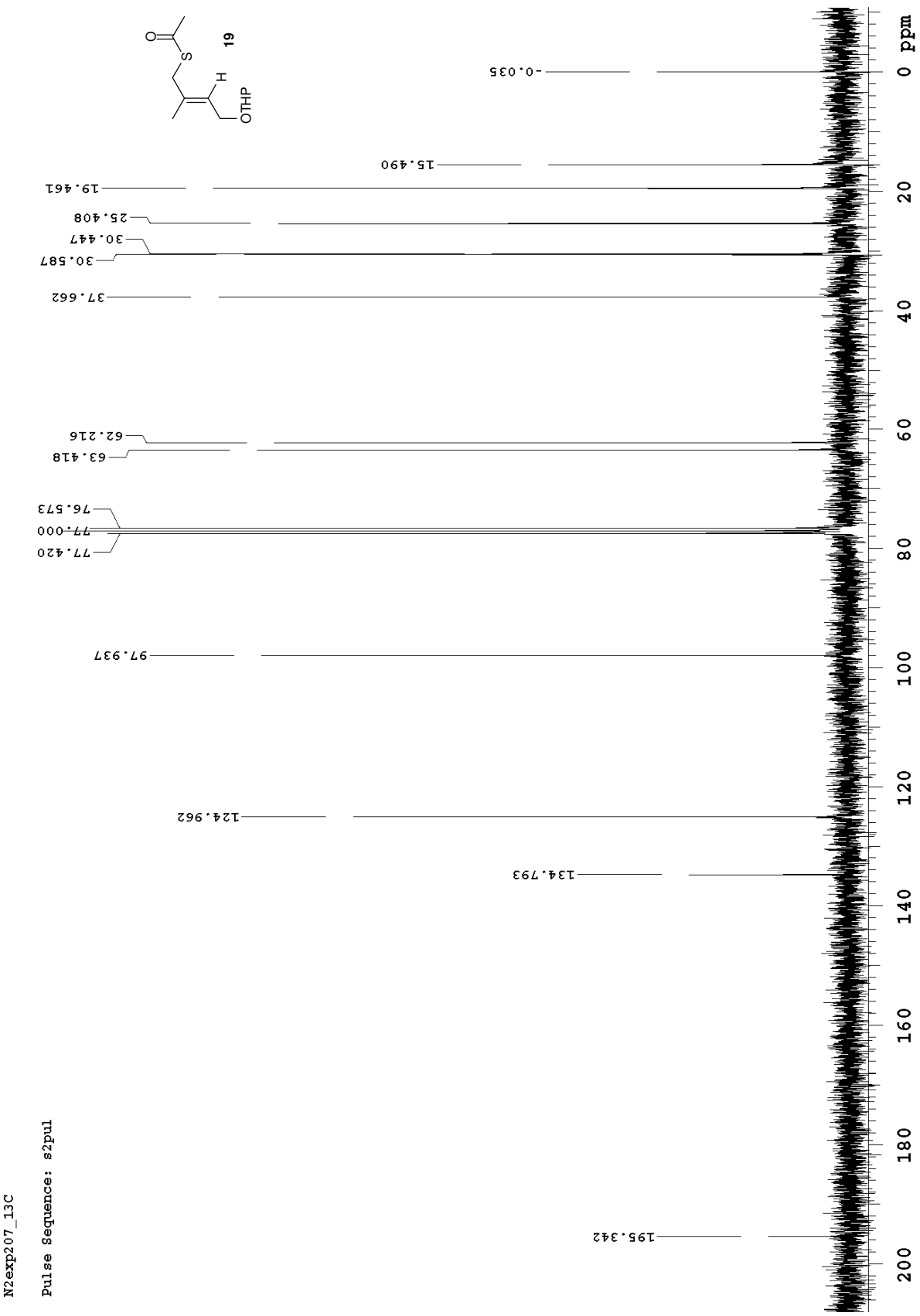

NHFmoc
H
OPP
18
-9.617
-9.441
-5.560
-5.384
2
-0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
-22
ppm

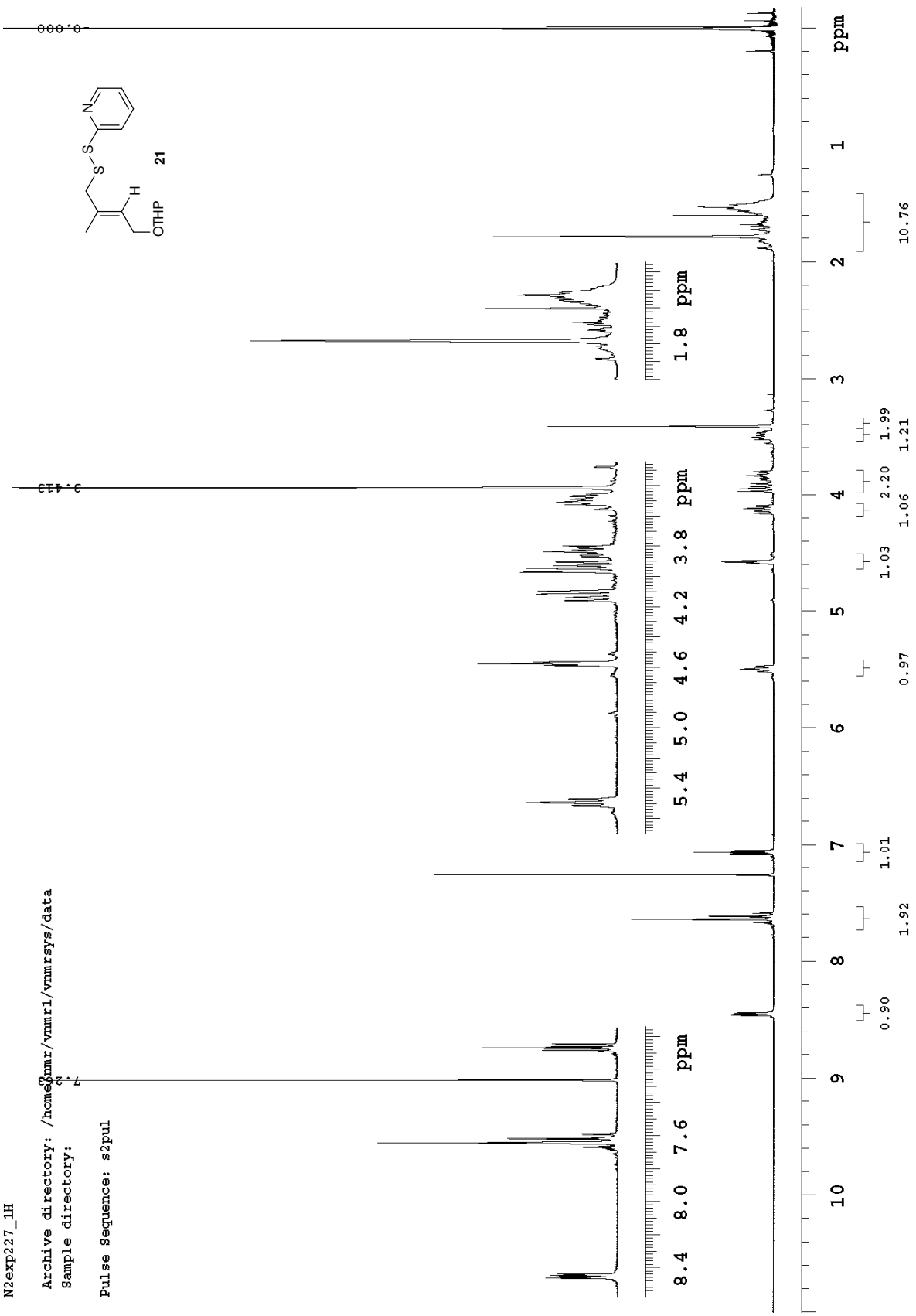

NH2
H
OPP
15
ppm
1.731
3.515
4.468
4.493
4.519
4.800
5.689
3.00
1.89
1.97
0.97

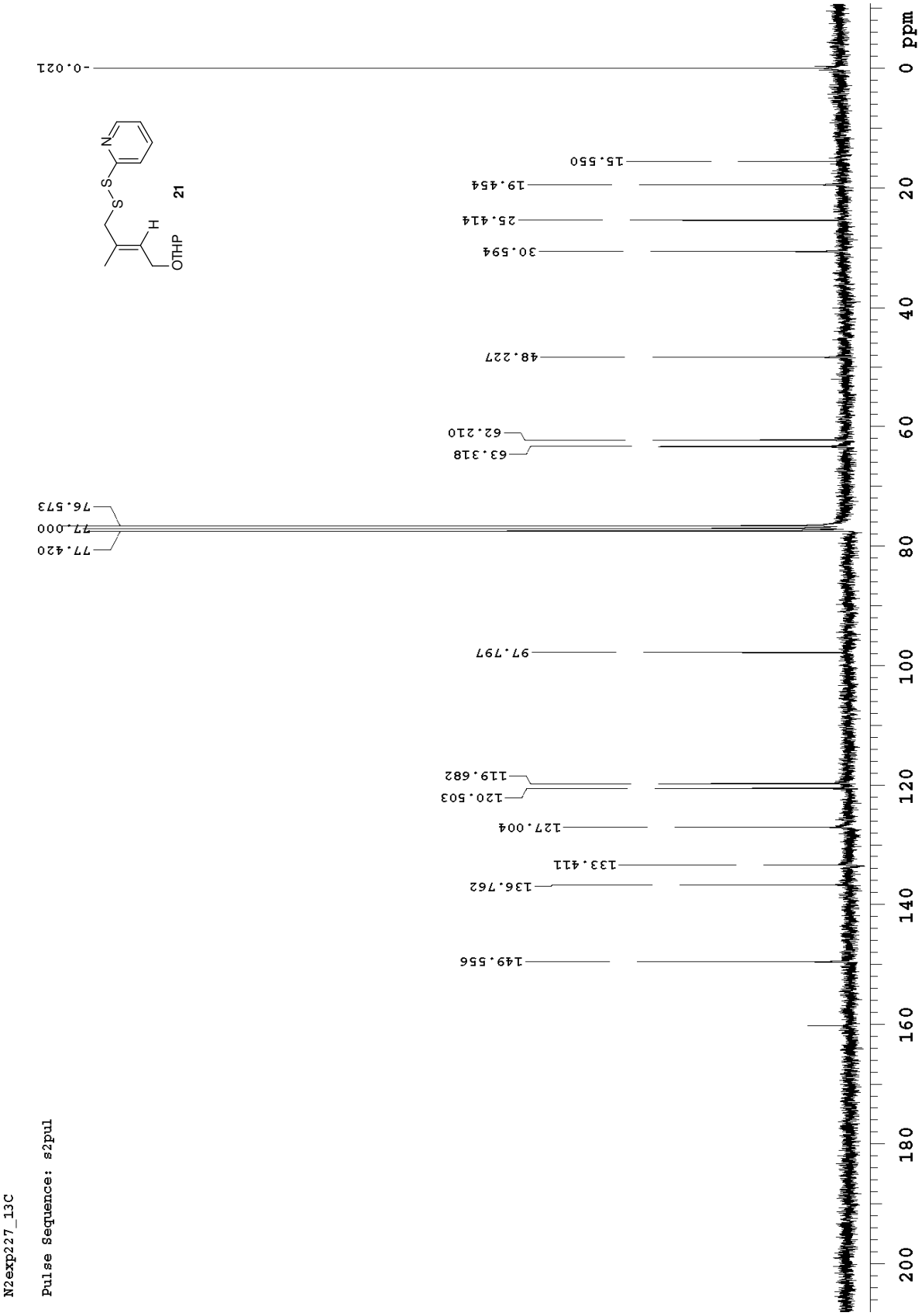

NH2
H
OPP
15
14.360
45.509
62.142
62.202
125.581
131.941
ppm
20
40
60
80
100
120
140
160
180
200

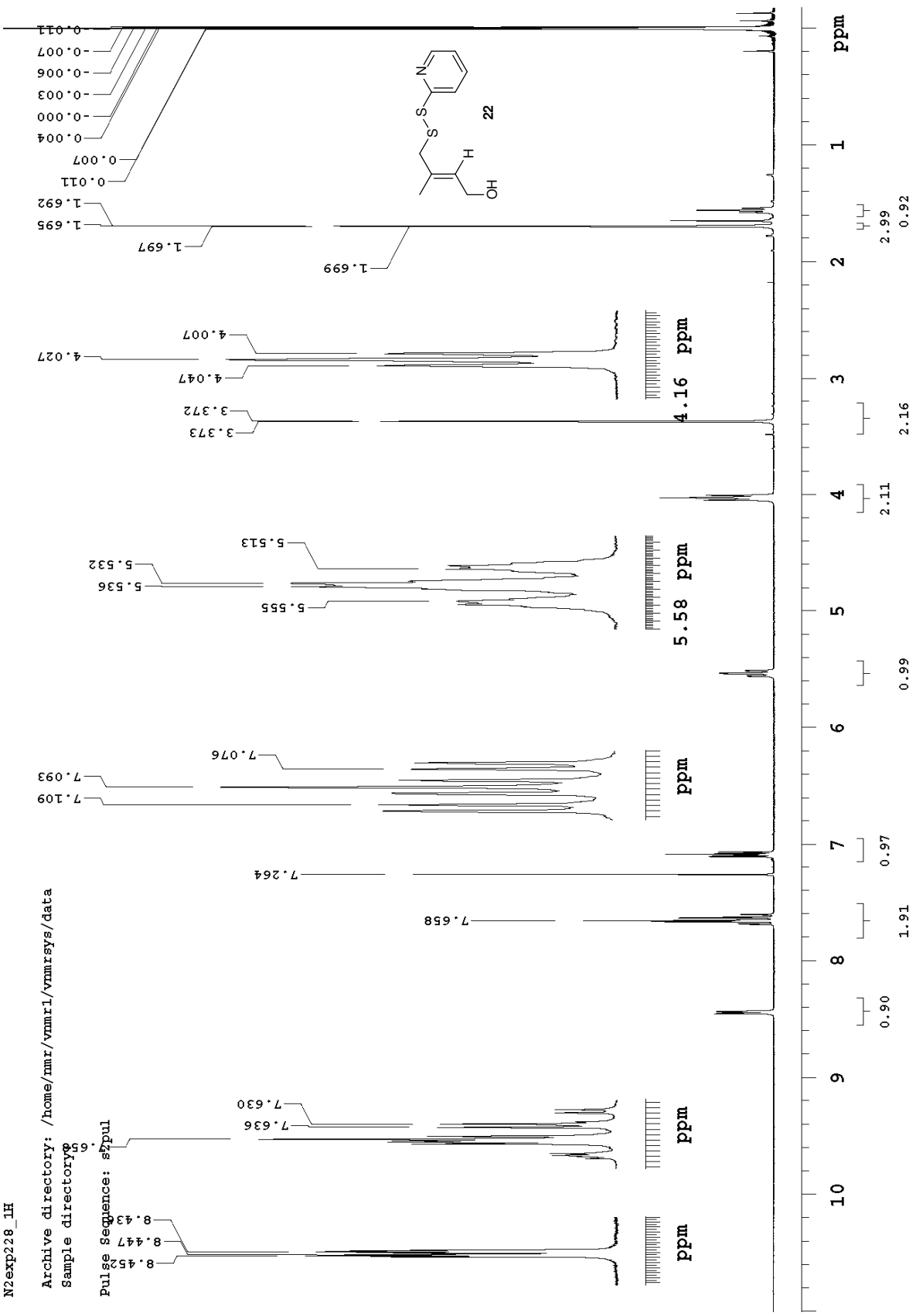

NH2
H
OPP
15
-9.496
-9.310
-5.561
-5.375
ppm
40
30
20
10
0
-10
-20
-30

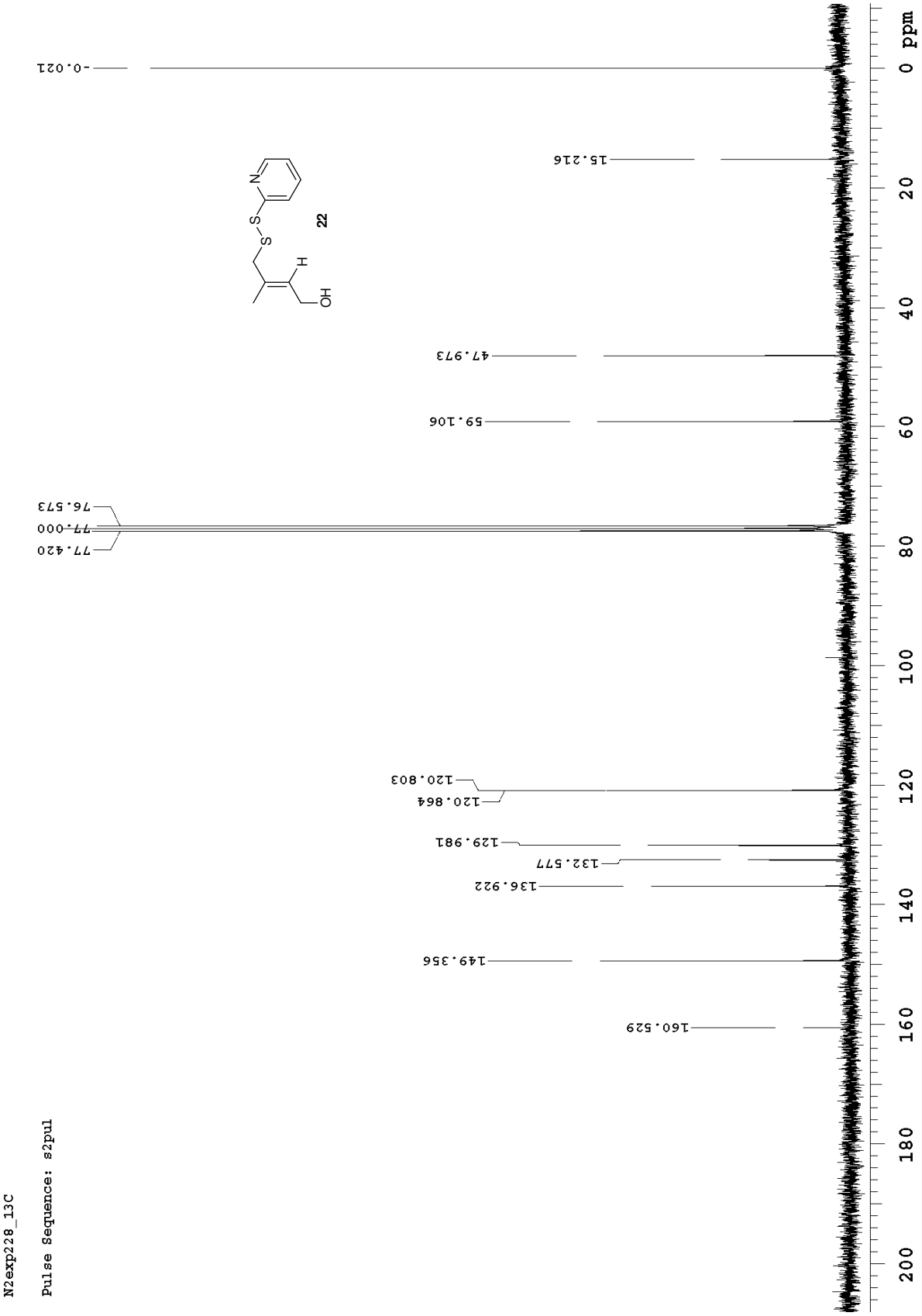

N2exp207_1H
Archive directory: /home/nmr/vnmr1/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
0.000
1.636
1.696
1.698
1.700
2.345
3.583
3.581
5.590
5.594
5.611
5.615
5.635
5.64 ppm
4.5 4.3 4.1 3.9 3.7 ppm
1.85 ppm
ppm
1.00
1.01
1.07
1.11
1.08
3.06
3.02
2.97
1.954.38
OTHP
H
S
O
19

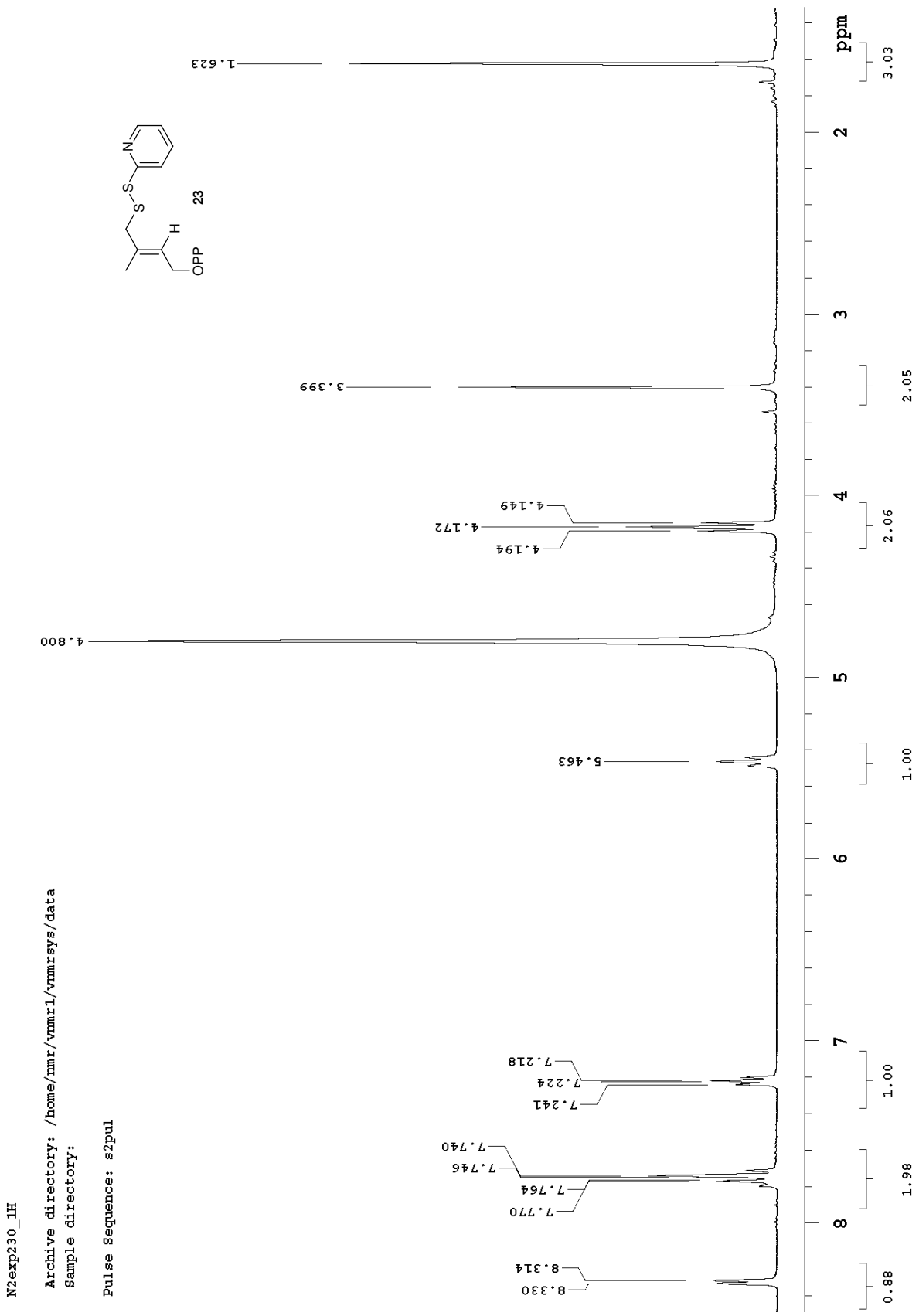

N2exp207_13C
Pulse Sequence: s2pul
195.342
134.793
124.962
97.937
77.420
77.000
76.573
63.418
62.216
37.662
30.587
30.447
25.408
19.461
15.490
-0.035
0 ppm
20
40
60
80
100
120
140
160
180
200
19
S
O
H
OTHP

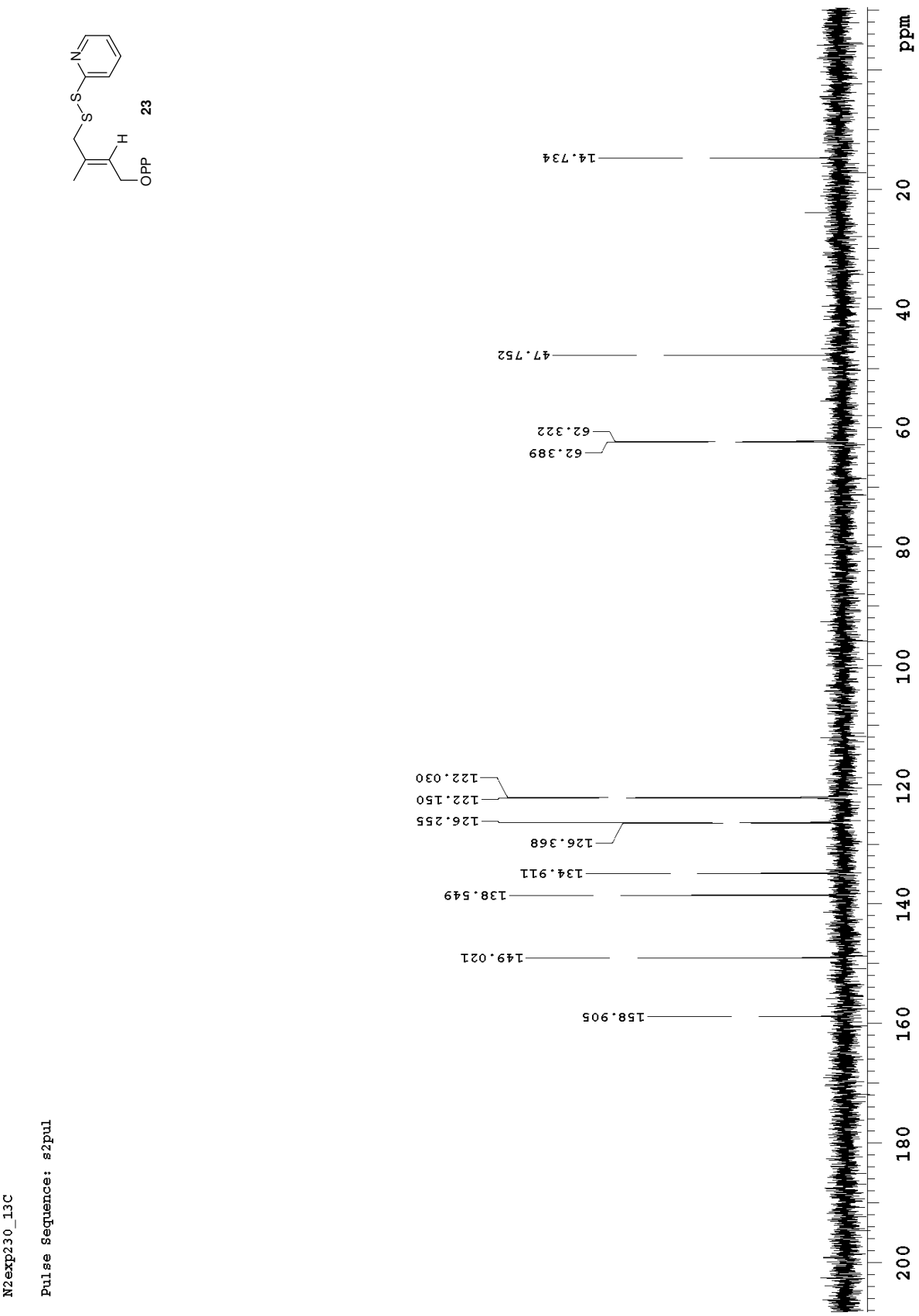

N2exp227_1H
Archive directory: /home/nmr/vnmr1/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
7.27
3.413
0.000
OTHP
21
8.4 8.0 7.6 ppm
5.4 5.0 4.6 4.2 3.8 ppm
1.8 ppm
ppm
0.90
1.92
1.01
0.97
1.03
1.06
2.20
1.21
1.99
10.76

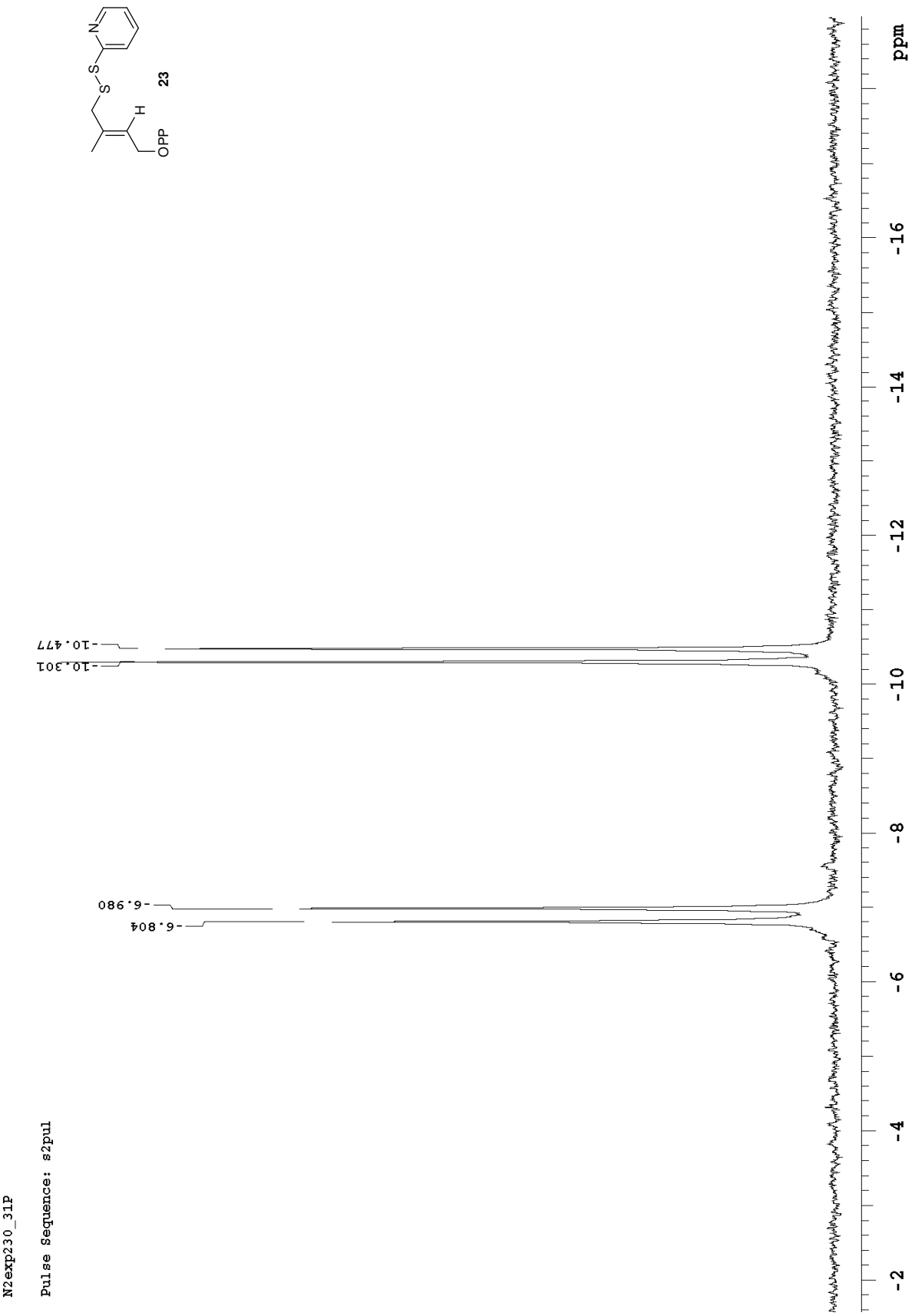

N2exp227_13C
Pulse Sequence: s2pul
-0.021
15.550
19.454
25.414
30.594
48.227
62.210
63.318
76.573
77.000
77.420
97.797
119.682
120.503
127.004
133.411
136.762
149.556
0 ppm
20
40
60
80
100
120
140
160
180
200
21
OTHP
H
S
S
N

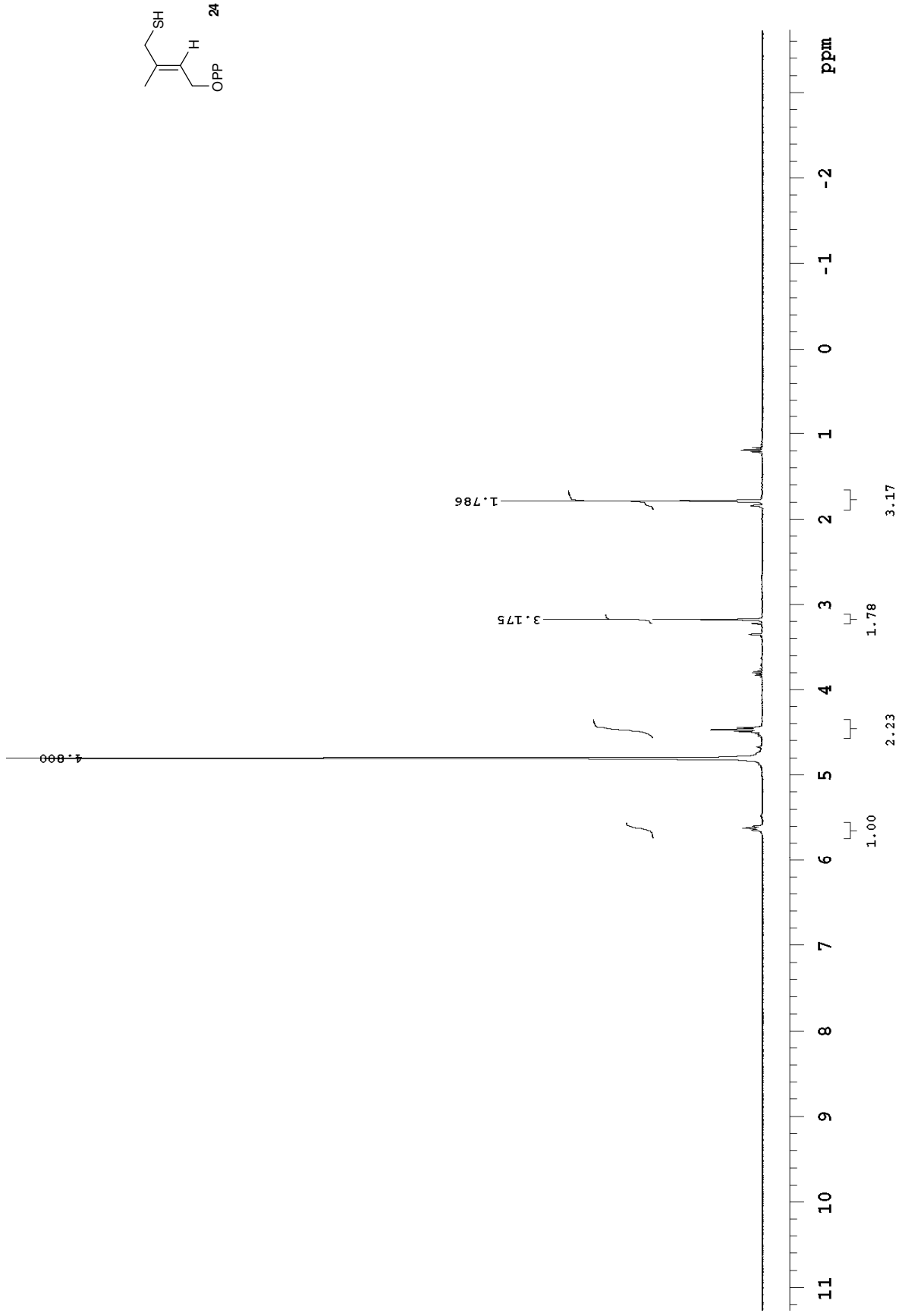

N2exp228_1H
Archive directory: /home/nmr/vnmr1/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
8.452
8.447
8.436
7.655
7.636
7.630
7.658
7.264
7.109
7.093
7.076
5.555
5.536
5.532
5.513
4.027
4.047
4.007
3.373
3.372
1.699
1.697
1.695
1.692
0.011
0.007
0.004
-0.000
-0.003
-0.006
-0.007
-0.011
ppm
4.16
5.58
2.99
0.92
2.16
2.11
0.99
0.97
1.91
0.90
22

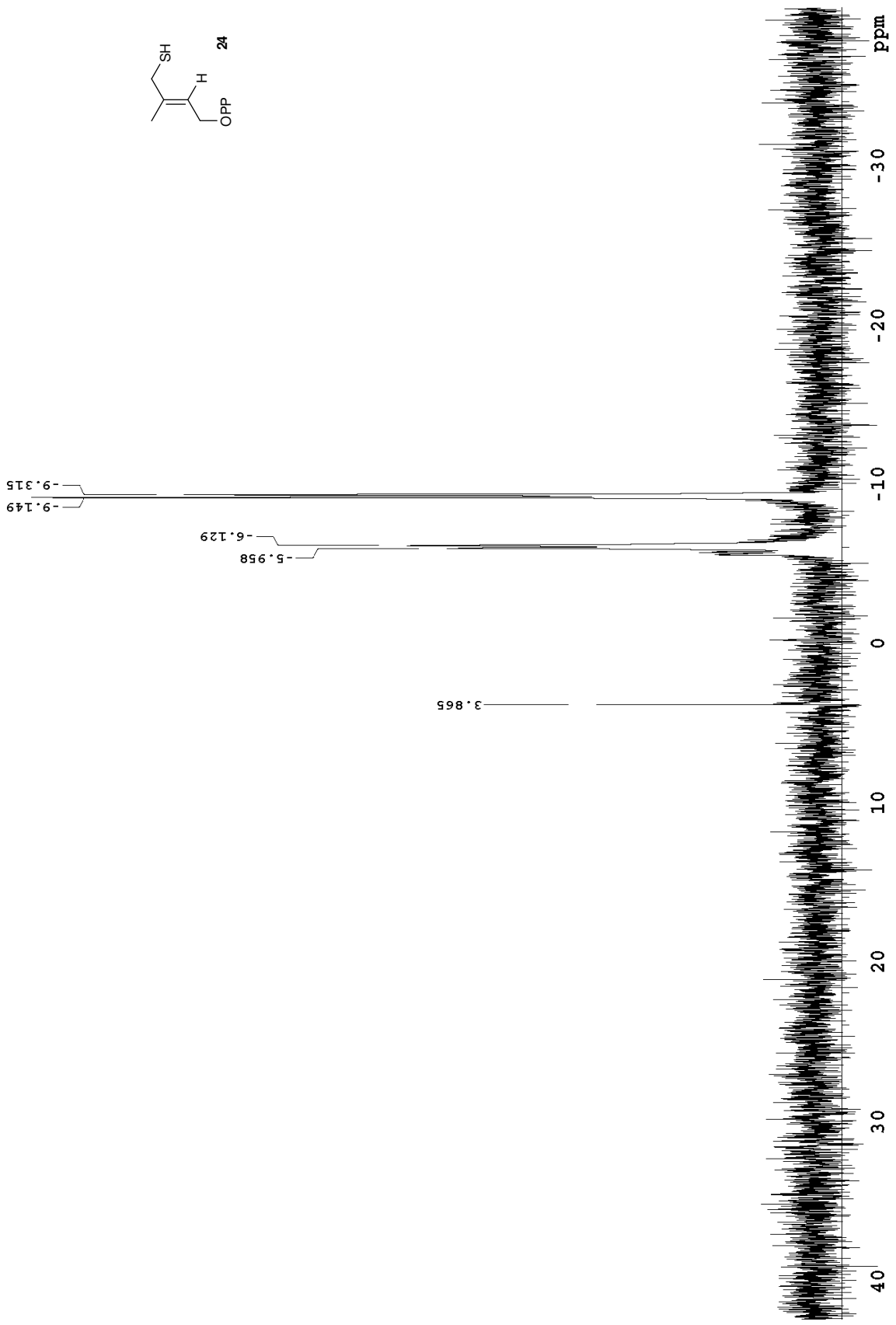

N2exp228_13C
Pulse Sequence: s2pul
-0.021
15.216
47.973
59.106
76.573
77.000
77.420
120.803
120.864
129.981
132.577
136.922
149.356
160.529
22
0 ppm
20
40
60
80
100
120
140
160
180
200

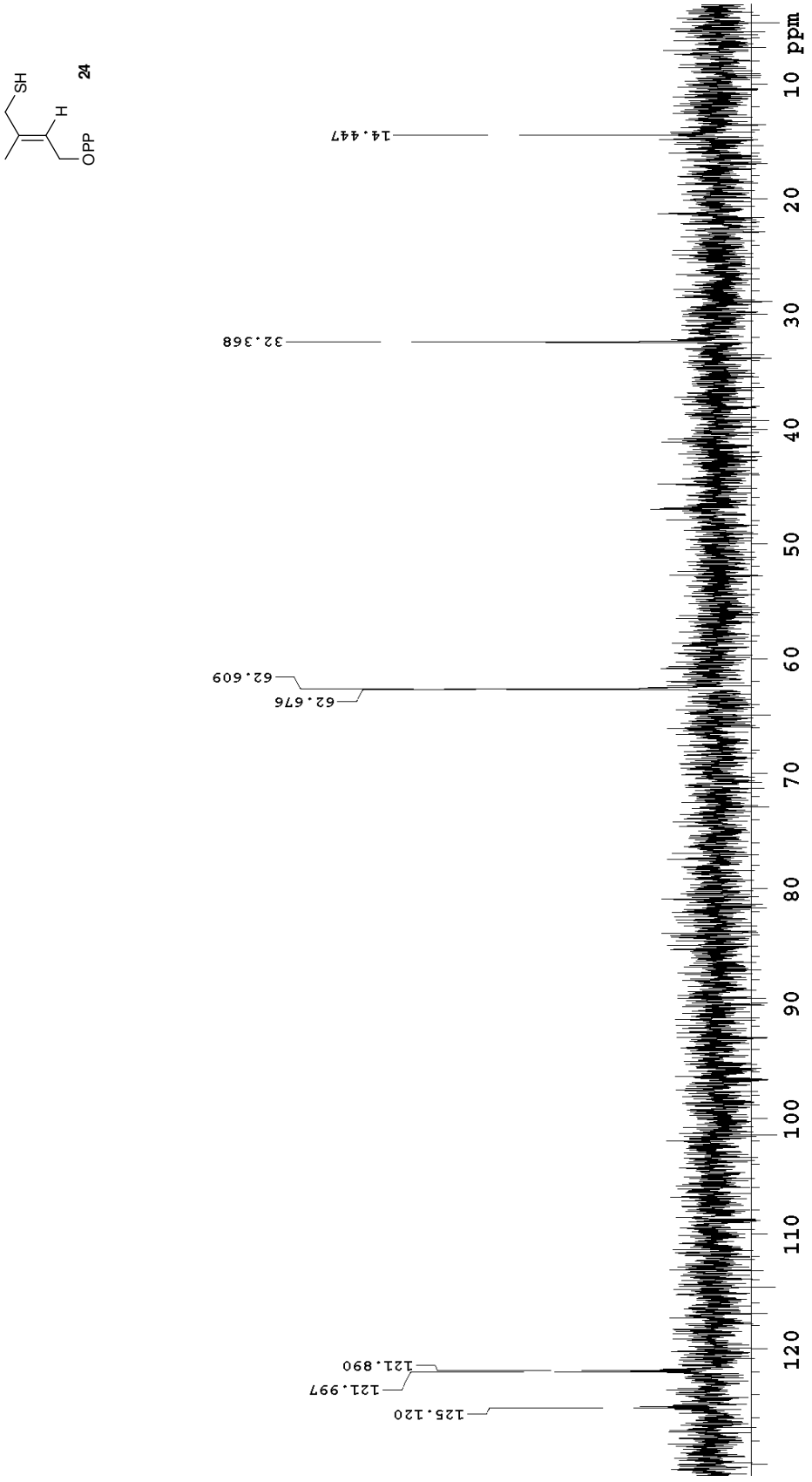

N2exp230_1H
Archive directory: /home/nmr/vnmr1/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
8.330
8.314
7.770
7.764
7.746
7.740
7.241
7.224
7.218
5.463
4.800
4.194
4.172
4.149
3.399
1.623
OPP
H
S
S
N
23
ppm
0.88
1.98
1.00
1.00
2.06
2.05
3.03


N2exp230_13C
Pulse Sequence: s2pul
N
S
S
H
OPP
23
158.905
149.021
138.549
134.911
126.368
126.255
122.150
122.030
62.389
62.322
47.752
14.734
ppm
200
180
160
140
120
100
80
60
40
20


N2exp230_31P
Pulse Sequence: s2pul
-10.477
-10.301
-6.804
-6.980
ppm
-2
-4
-6
-8
-10
-12
-14
-16
N
S
S
H
OPP
23


SH
H
OPP
24
ppm
1.786
3.175
4.800
1.00
2.23
1.78
3.17

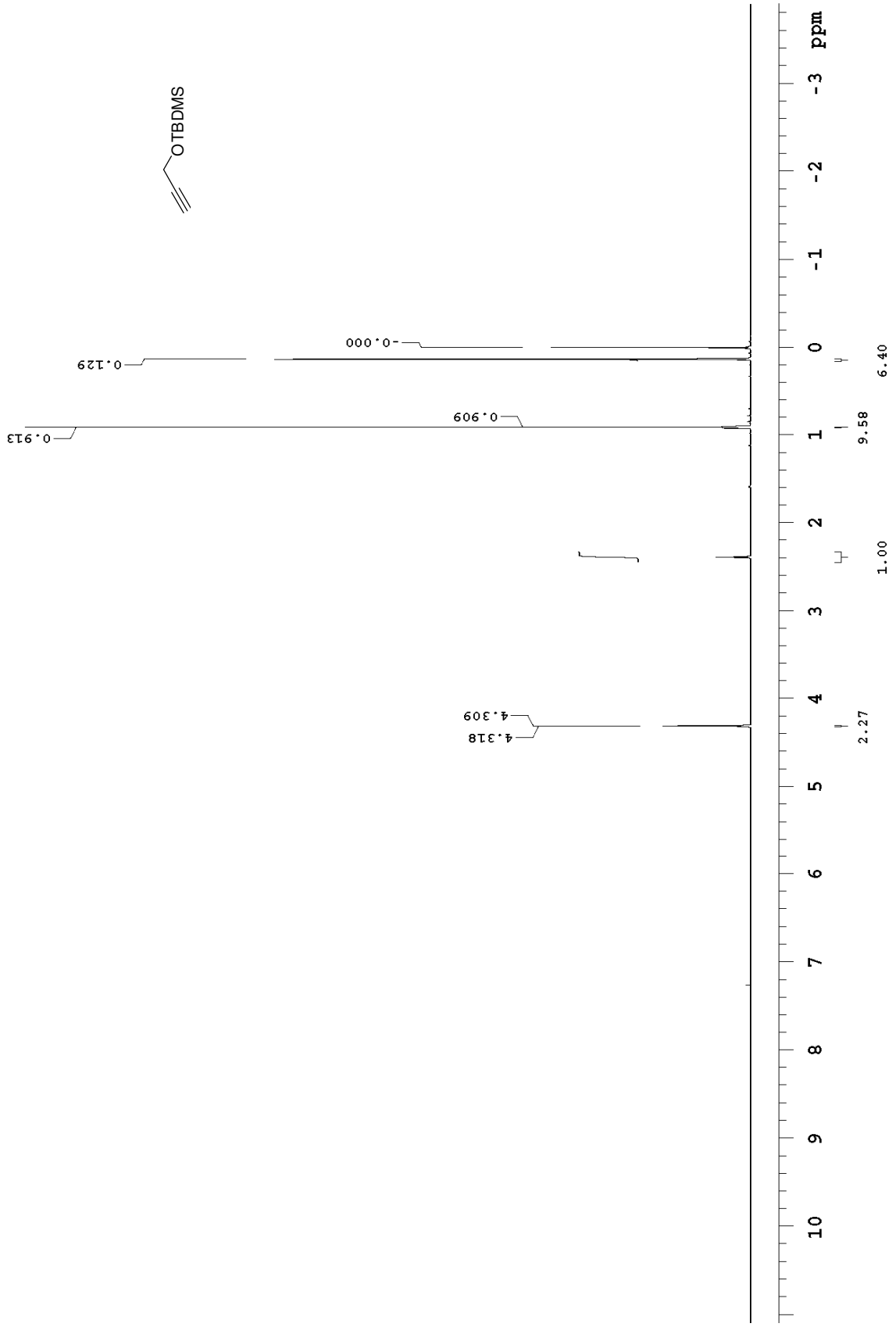

24
SH
H
OPP
-9.315
-9.149
-6.129
-5.958
3.865
ppm
40
30
20
10
0
-10
-20
-30

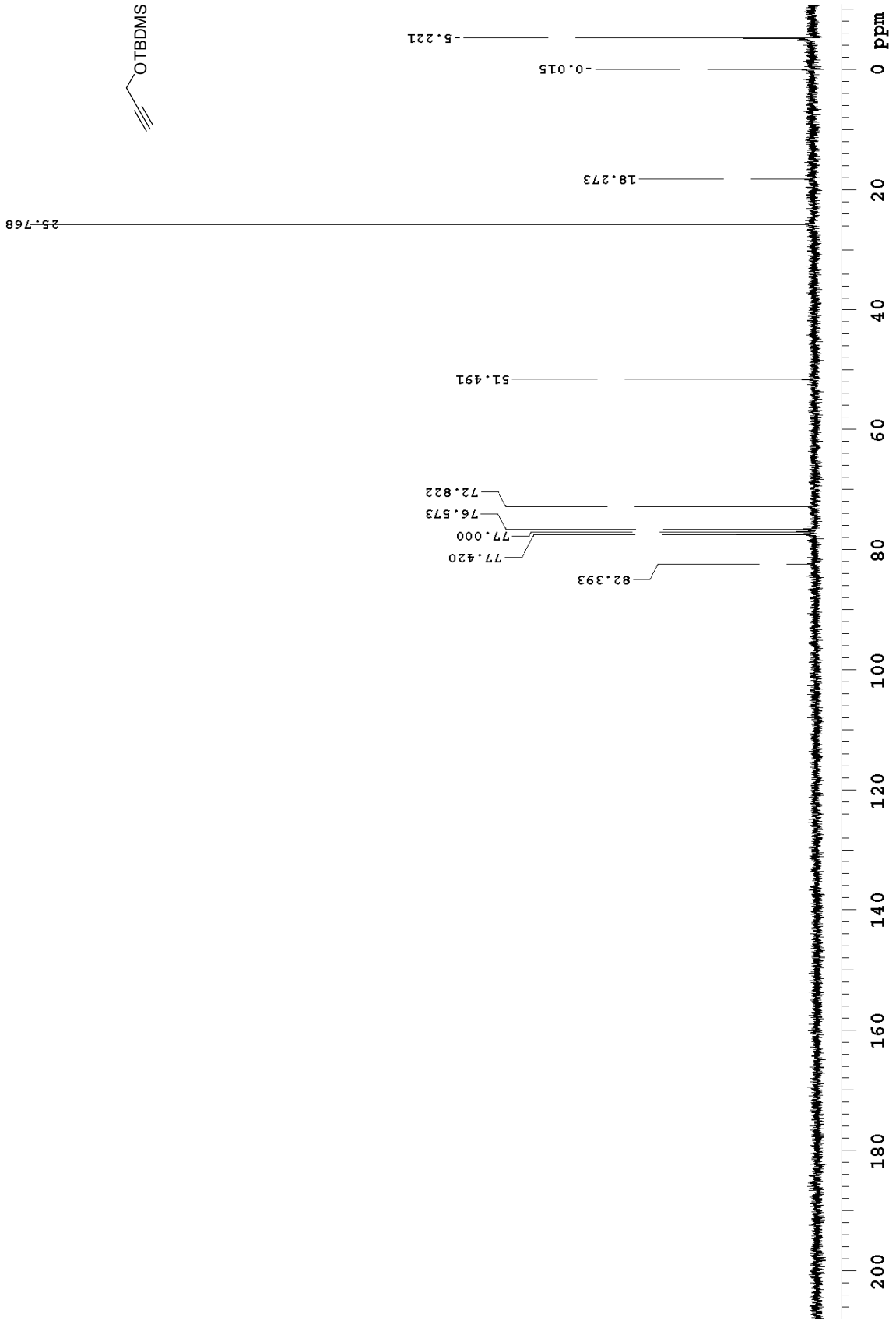

SH
H
OPP
24
14.447
32.368
62.609
62.676
121.890
121.997
125.120
10 ppm
20
30
40
50
60
70
80
90
100
110
120

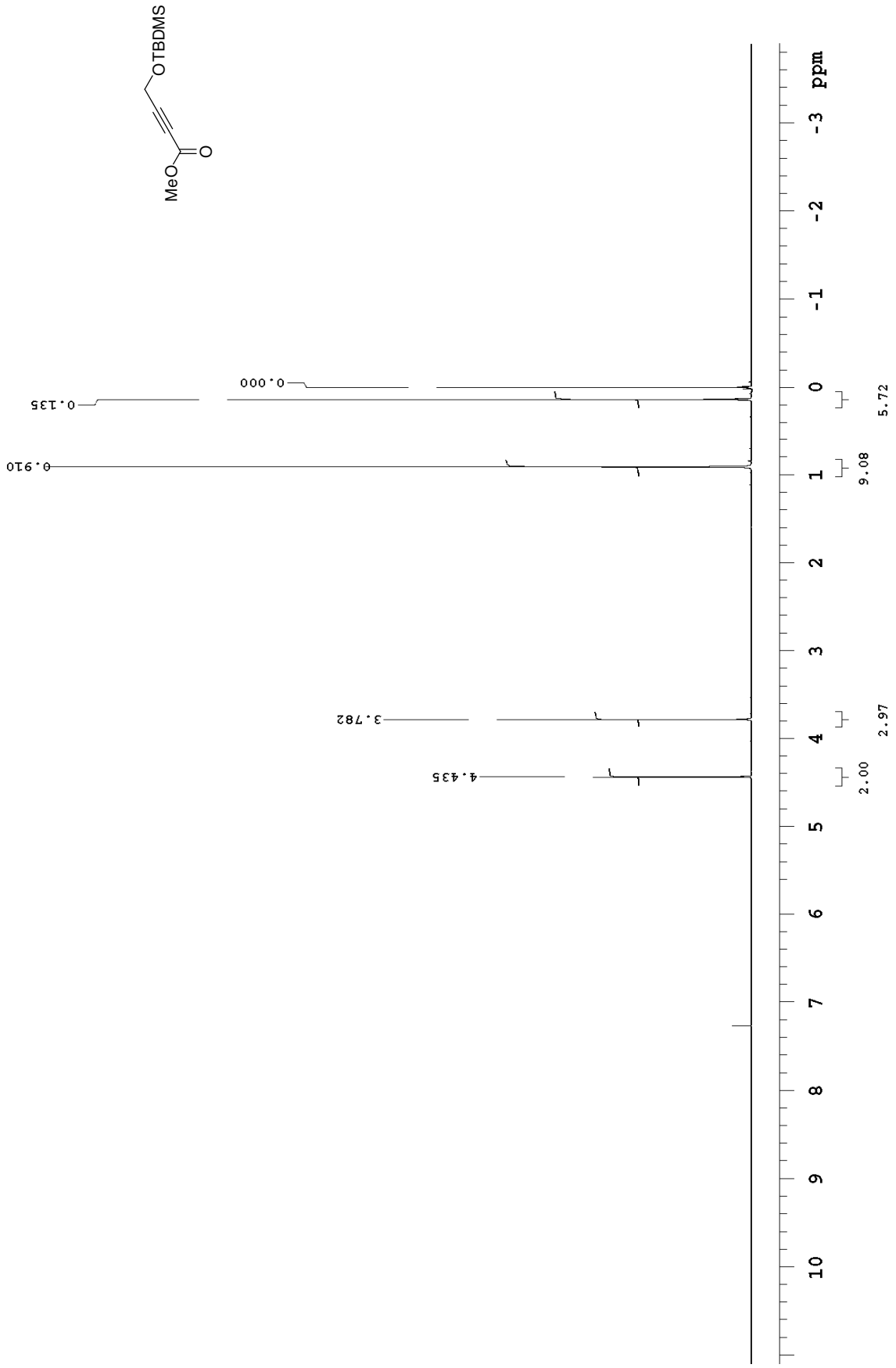
APPENDIX B

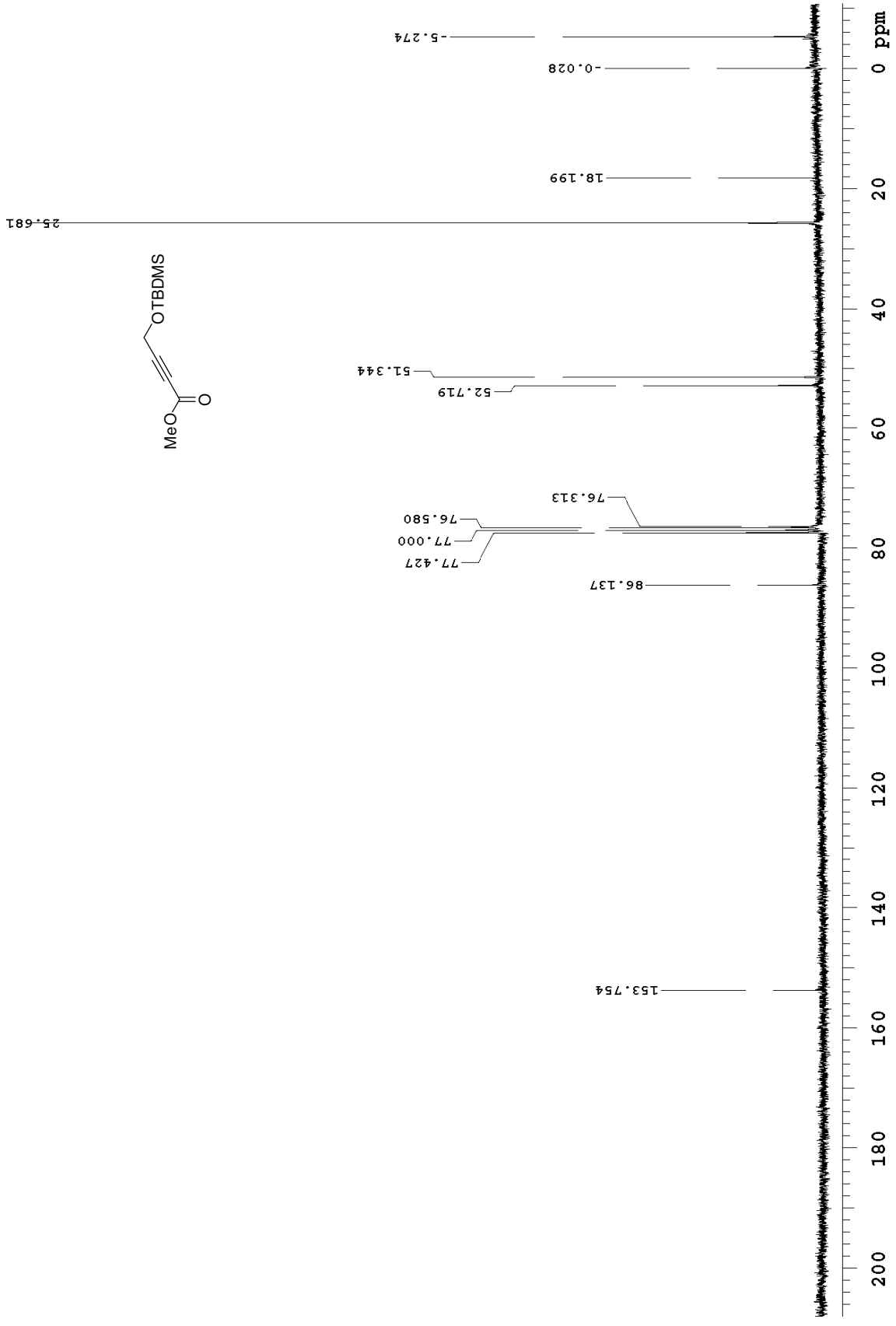
^{1}H, ^{13}C, ^{31}P NMR SPECTRA OF SELECTED COMPOUNDS

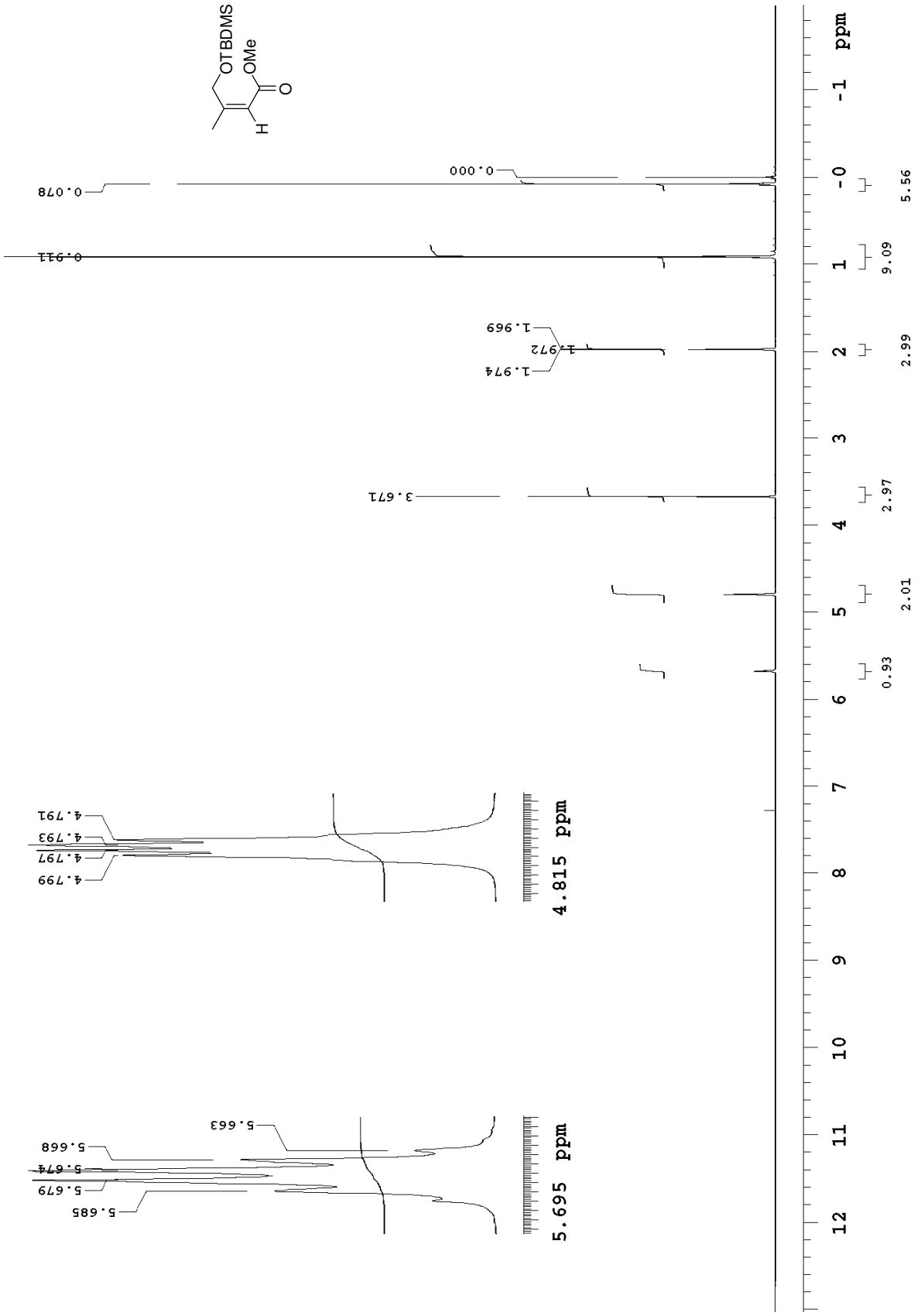
FOR CHAPTER 3

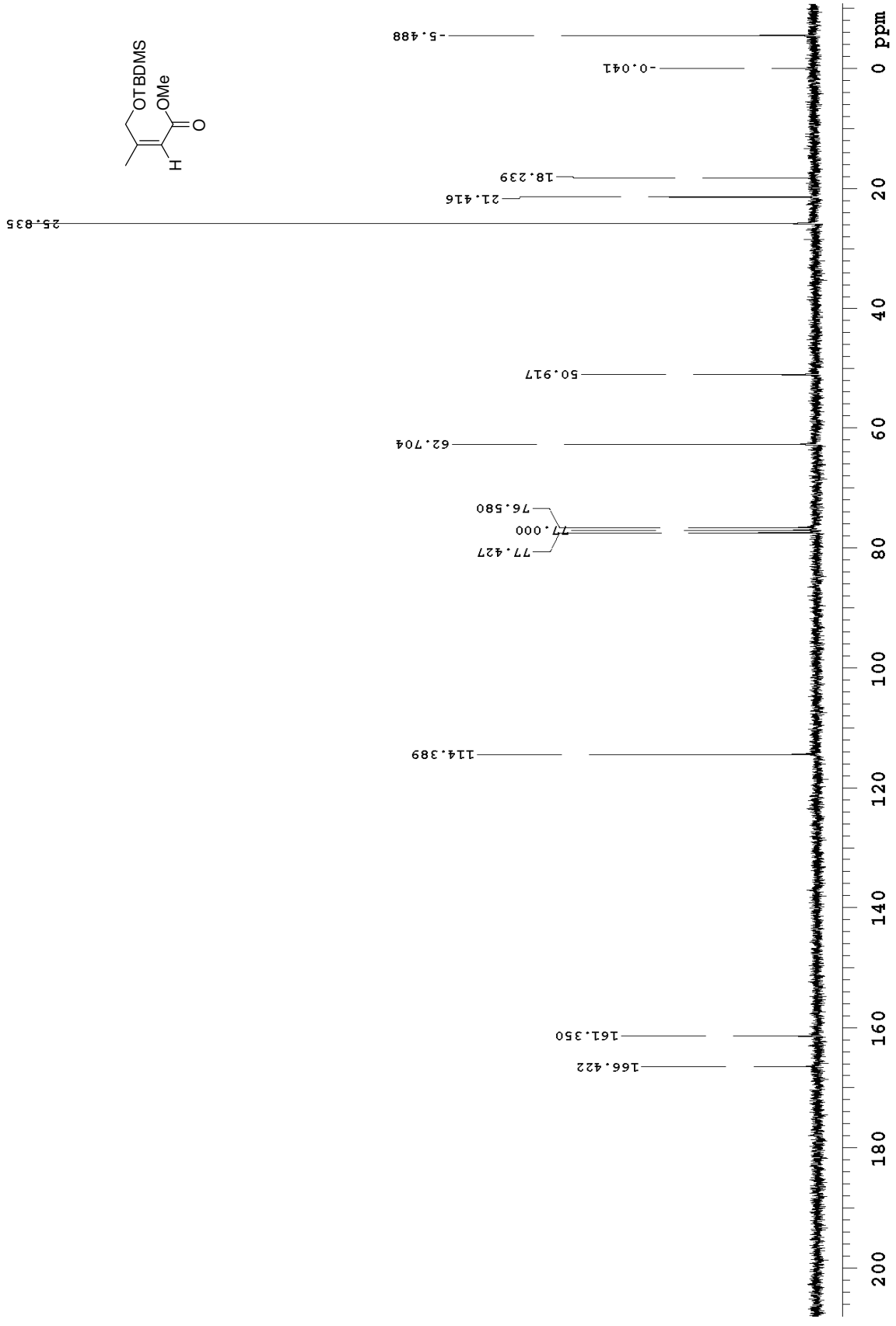

OTBDMS
0.913
0.909
0.129
-0.000
4.318
4.309
ppm
6.40
9.58
1.00
2.27

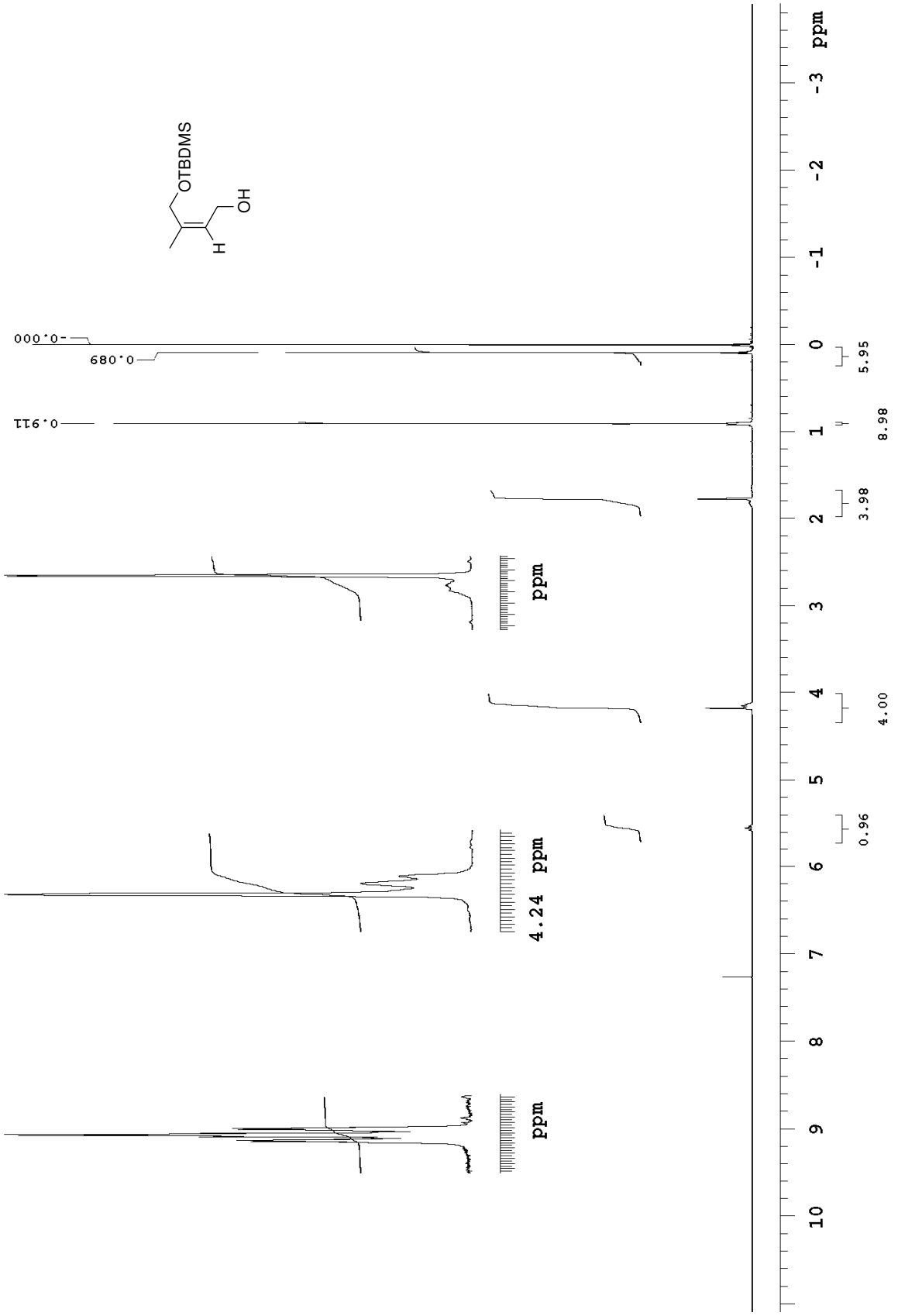

OTBDMS
-5.221
-0.015
18.273
25.768
51.491
72.822
76.573
77.000
77.420
82.393
0 ppm
20
40
60
80
100
120
140
160
180
200

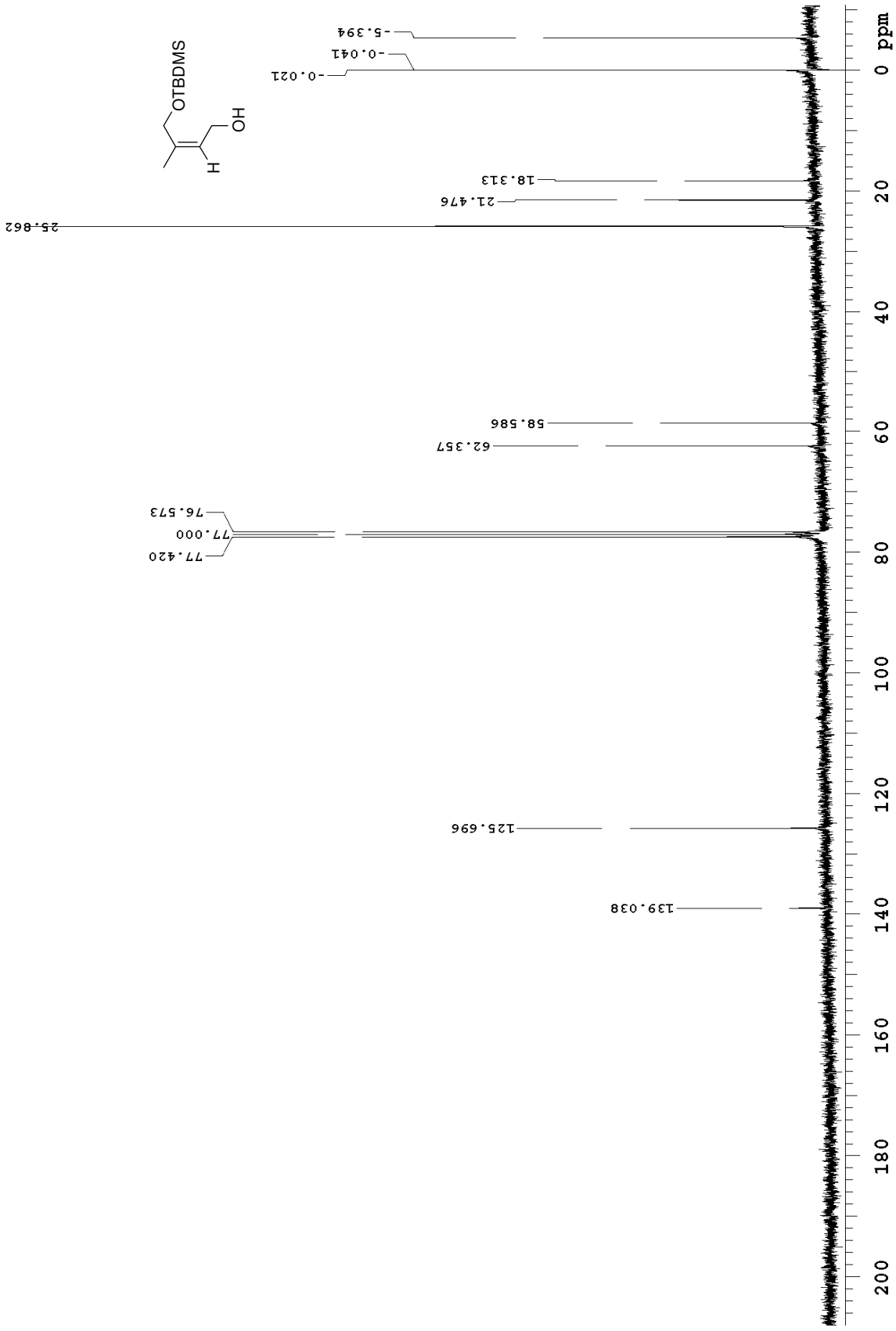

OTBDMS
MeO
O
0.000
0.135
0.910
3.782
4.435
5.72
9.08
2.97
2.00
ppm

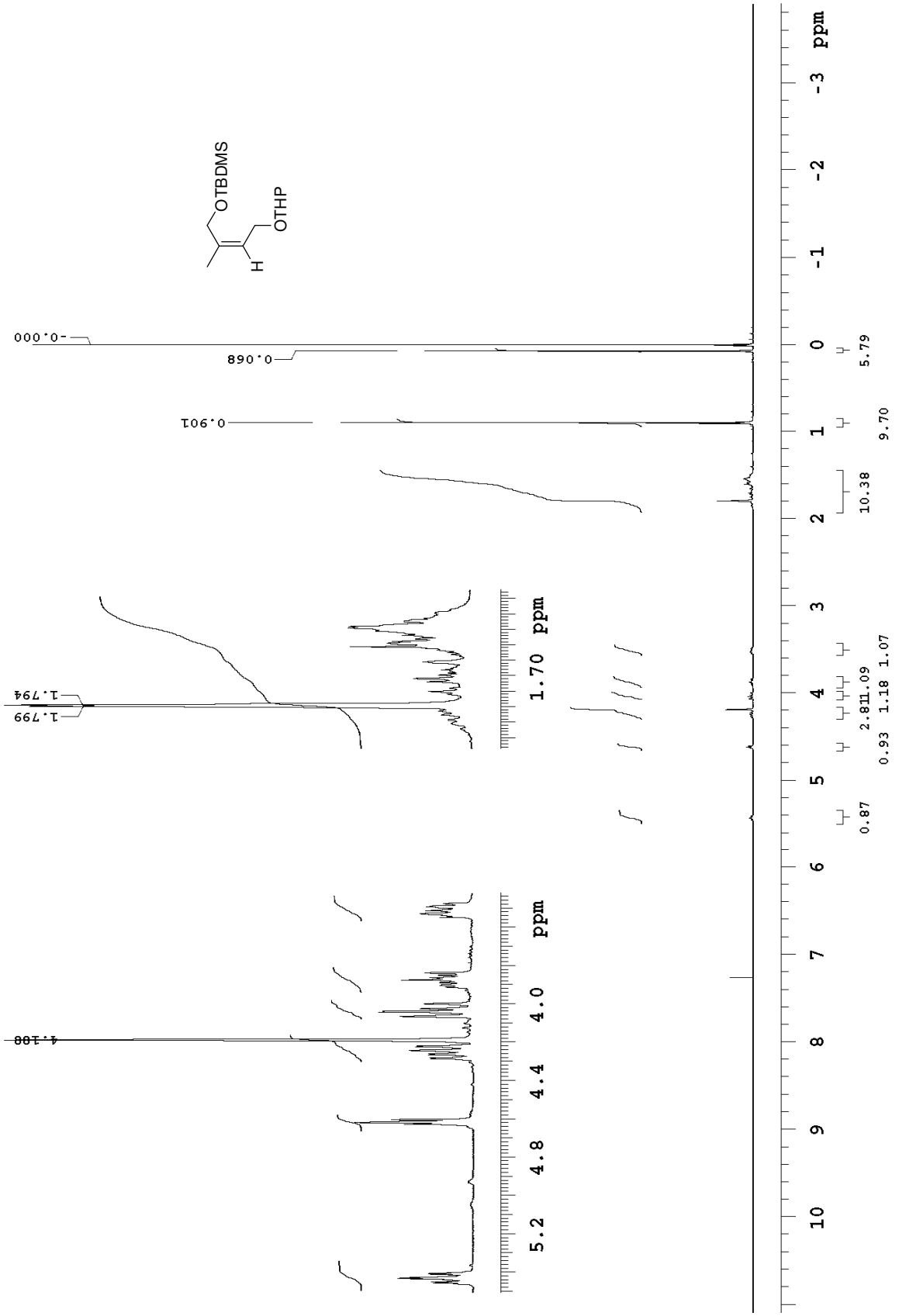

153.754
86.137
77.427
77.000
76.580
76.313
52.719
51.344
25.681
18.199
-0.028
-5.274
OTBDMS
MeO
O
0 ppm
20
40
60
80
100
120
140
160
180
200

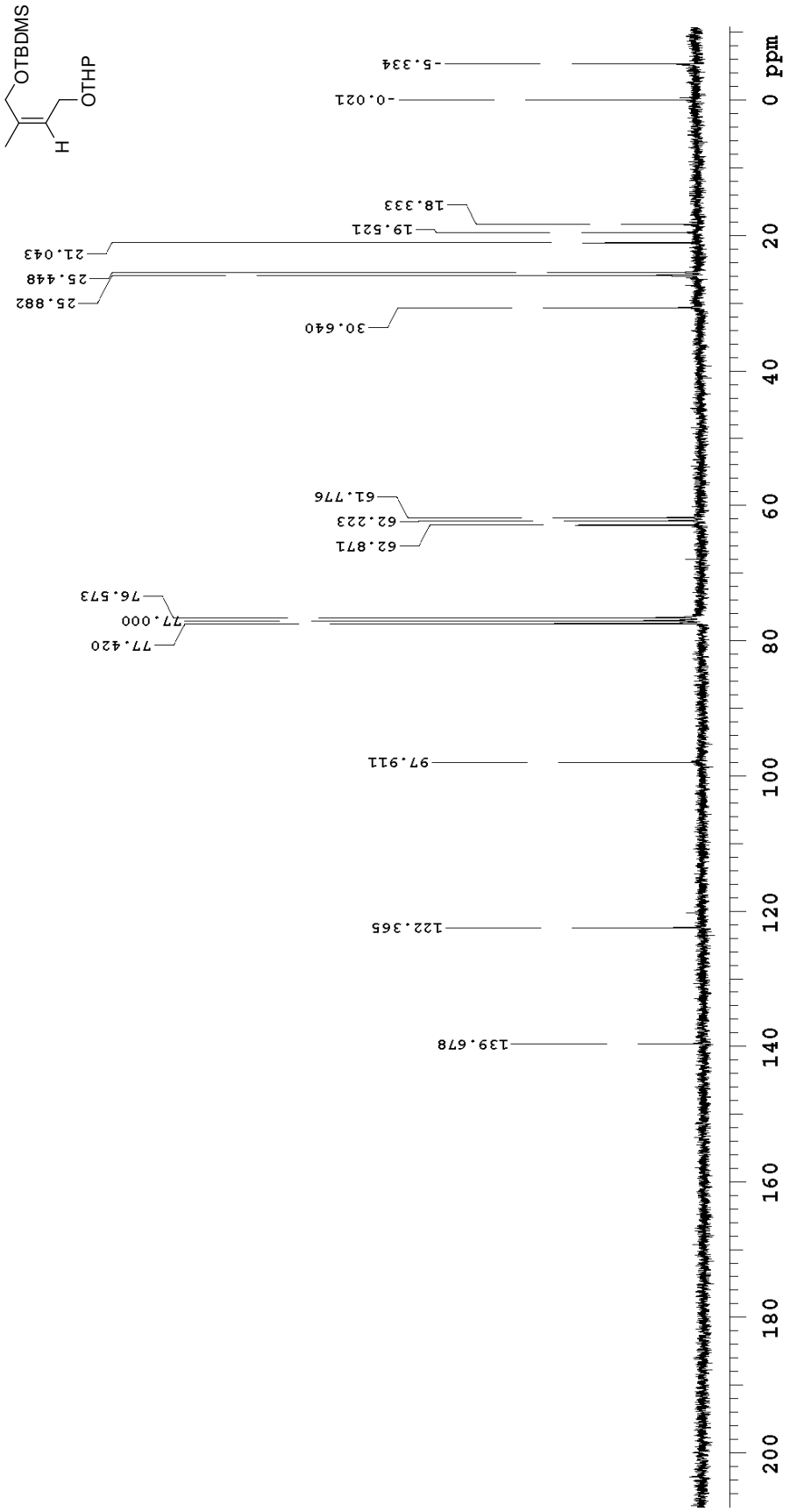

OTBDMS
OMe
O
H
0.078
0.000
0.911
1.969
1.972
1.974
3.671
4.799
4.797
4.793
4.791
4.815 ppm
5.685
5.679
5.674
5.668
5.663
5.695 ppm
ppm
5.56
9.09
2.99
2.97
2.01
0.93

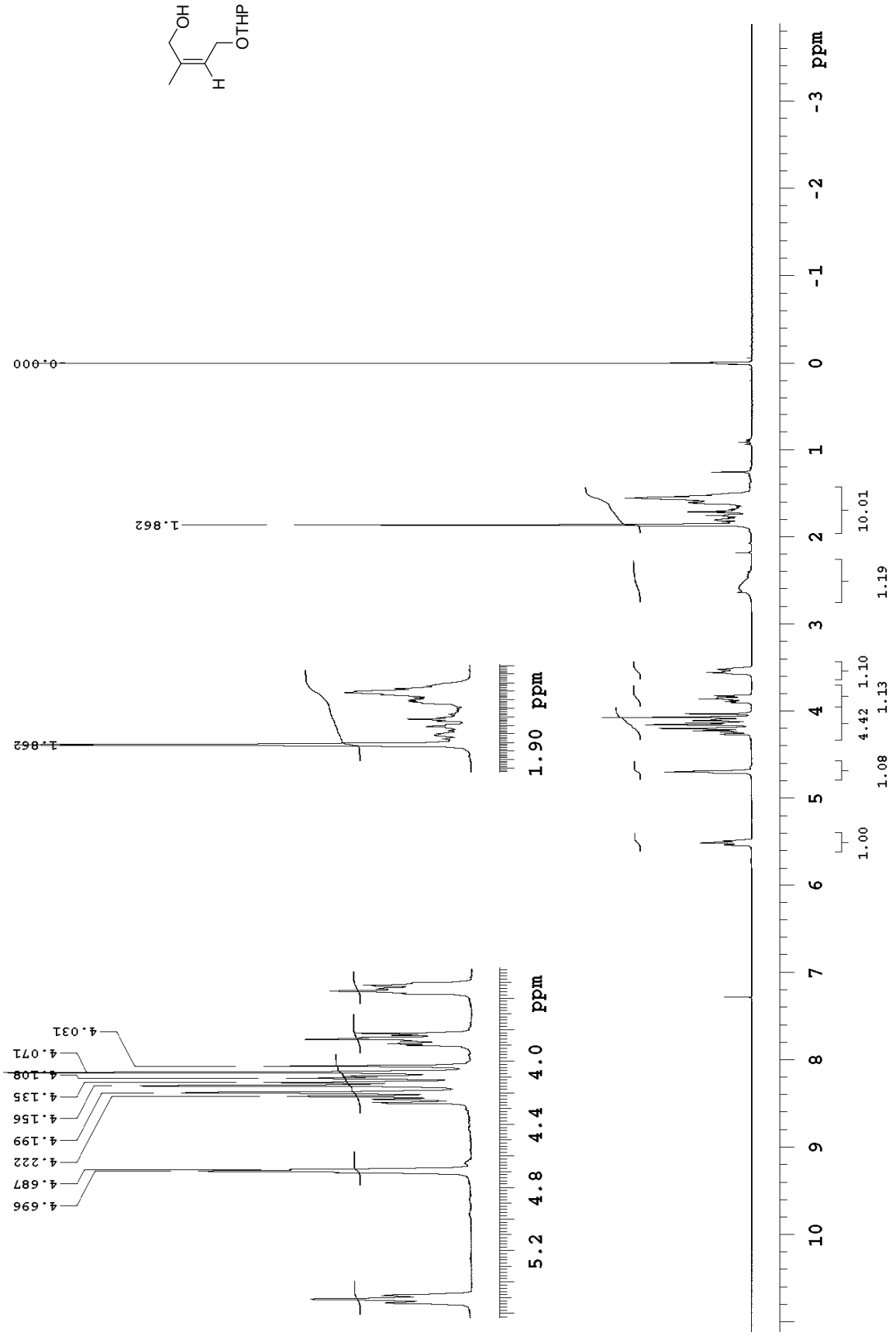

OTBDMS
OMe
O
H
-5.488
-0.041
18.239
21.416
25.835
50.917
62.704
76.580
77.000
77.427
114.389
161.350
166.422
0 ppm
20
40
60
80
100
120
140
160
180
200

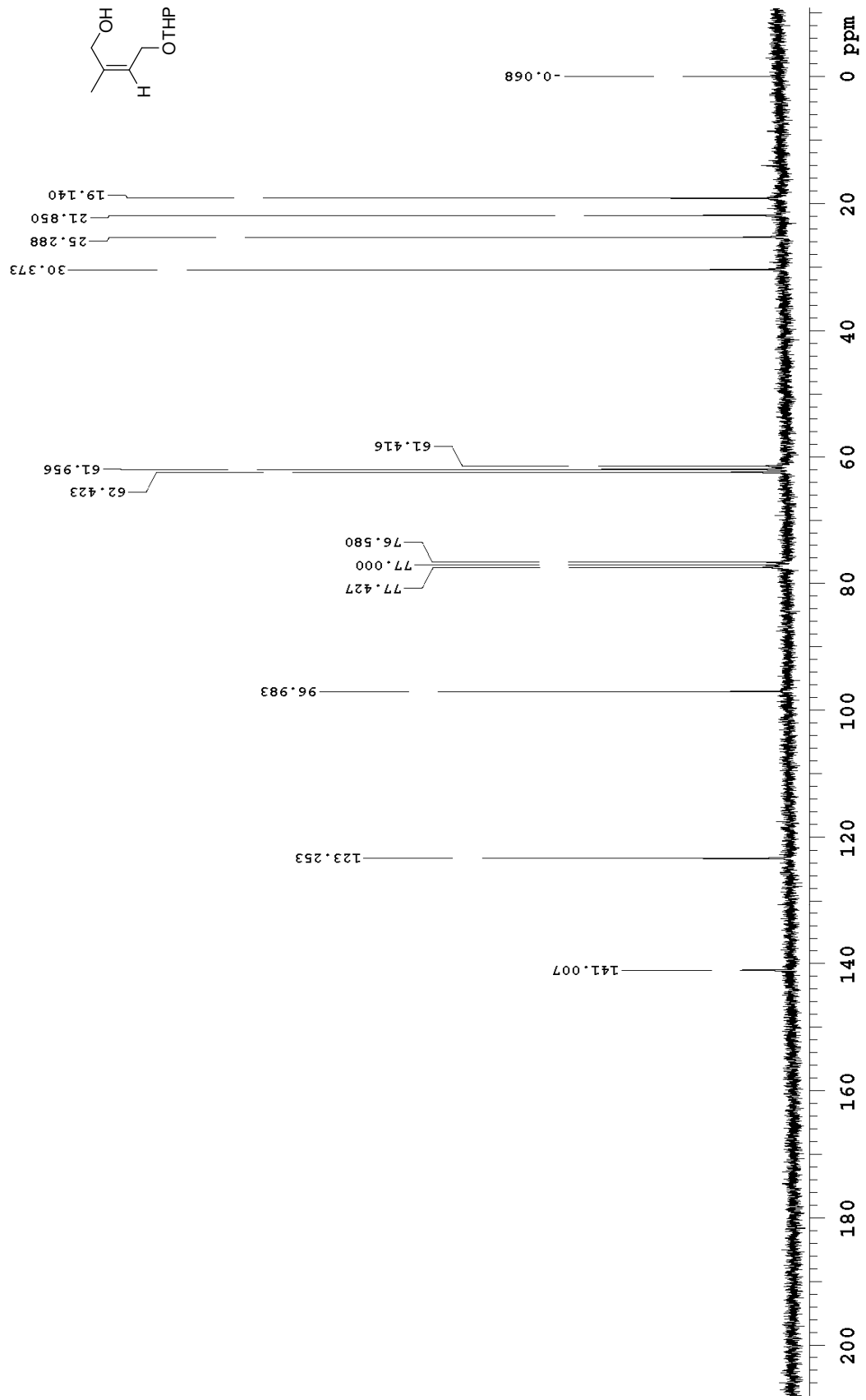

OTBDMS
OH
H
-0.000
0.089
0.911
5.95
8.98
3.98
4.00
0.96
4.24 ppm
ppm
ppm
10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 ppm

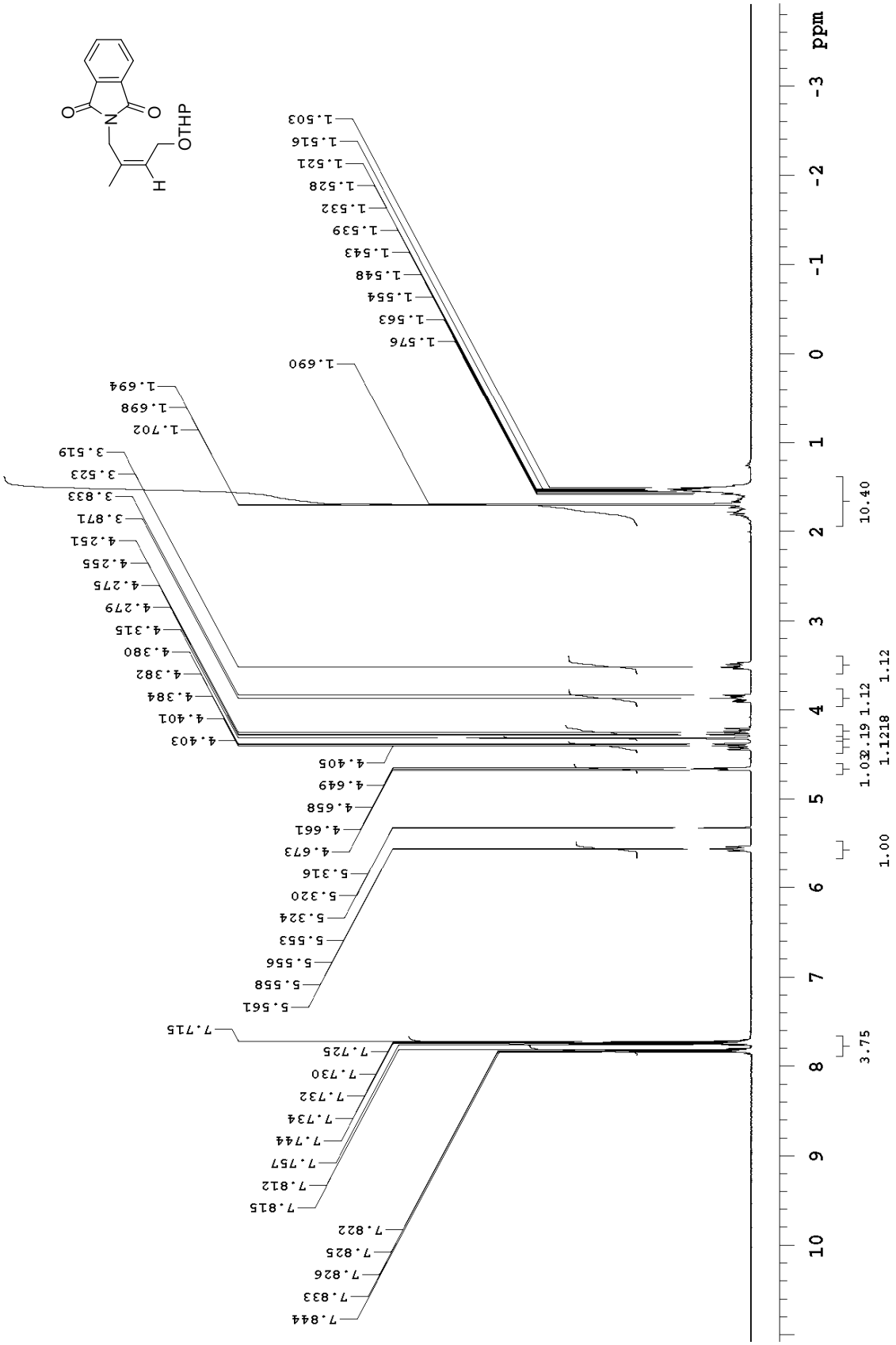

-5.394
-0.041
-0.021
18.313
21.476
25.862
58.586
62.357
76.573
77.000
77.420
125.696
139.038
0 ppm
20
40
60
80
100
120
140
160
180
200
OTBDMS
OH
H

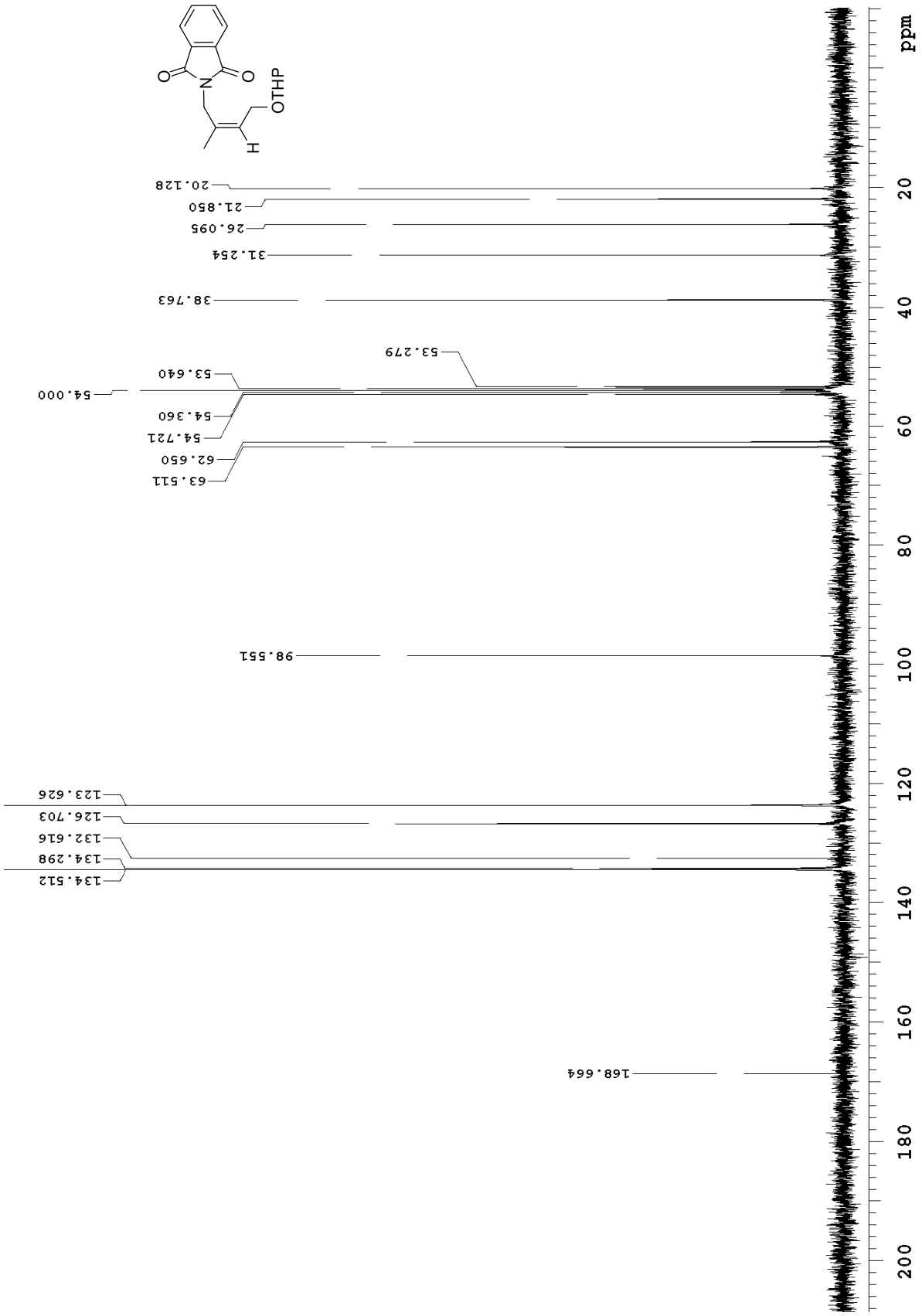

OTBDMS
OTHP
H
-0.000
0.068
0.901
1.799
1.794
1.188
1.70 ppm
5.2 4.8 4.4 4.0 ppm
10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 ppm
0.87
2.81
1.09
0.93
1.18
1.07
10.38
5.79
9.70

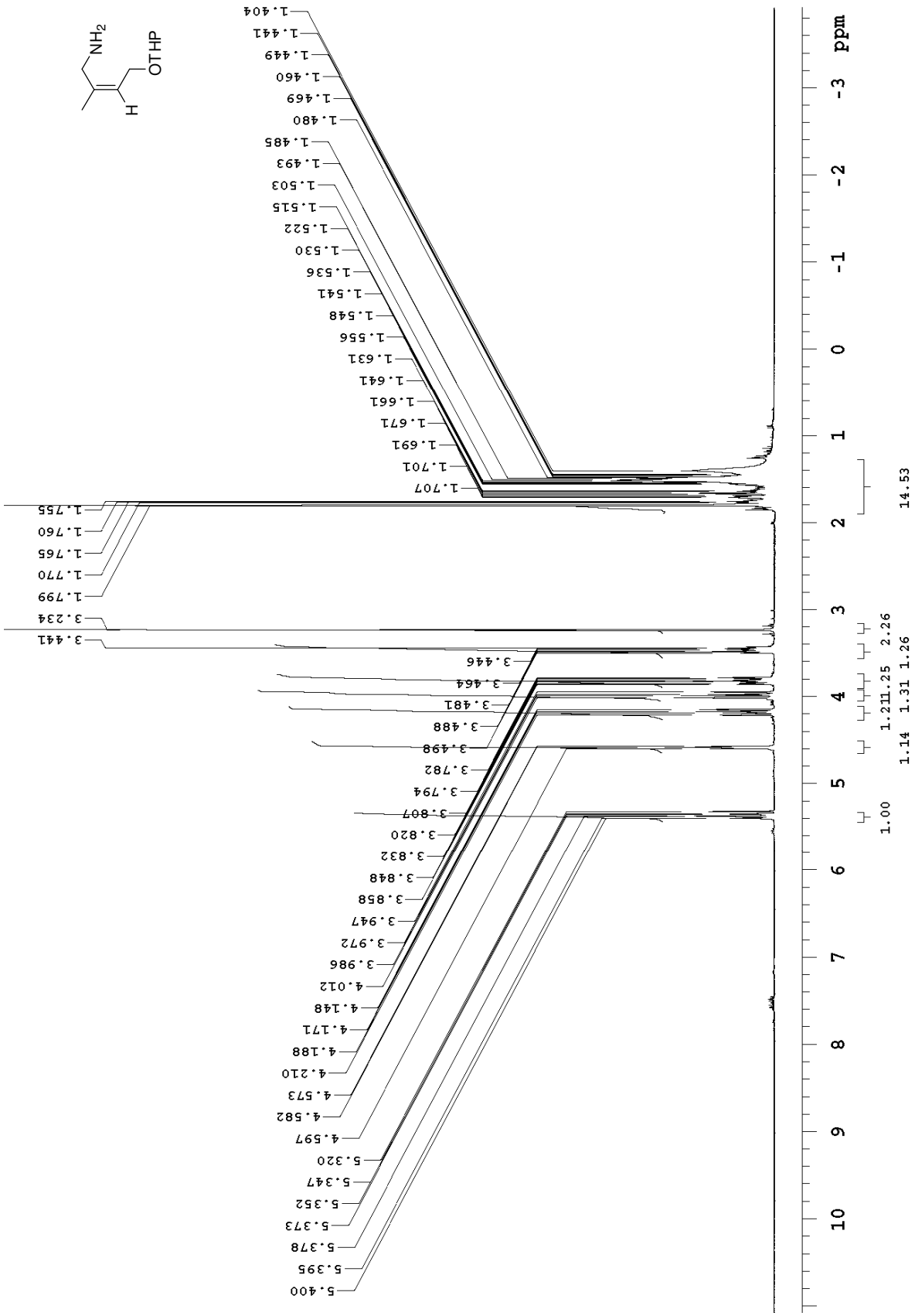

OTBDMS
OTHP
H
-5.334
-0.021
18.333
19.521
21.043
25.448
25.882
30.640
61.776
62.223
62.871
76.573
77.000
77.420
97.911
122.365
139.678
0 ppm
20
40
60
80
100
120
140
160
180
200

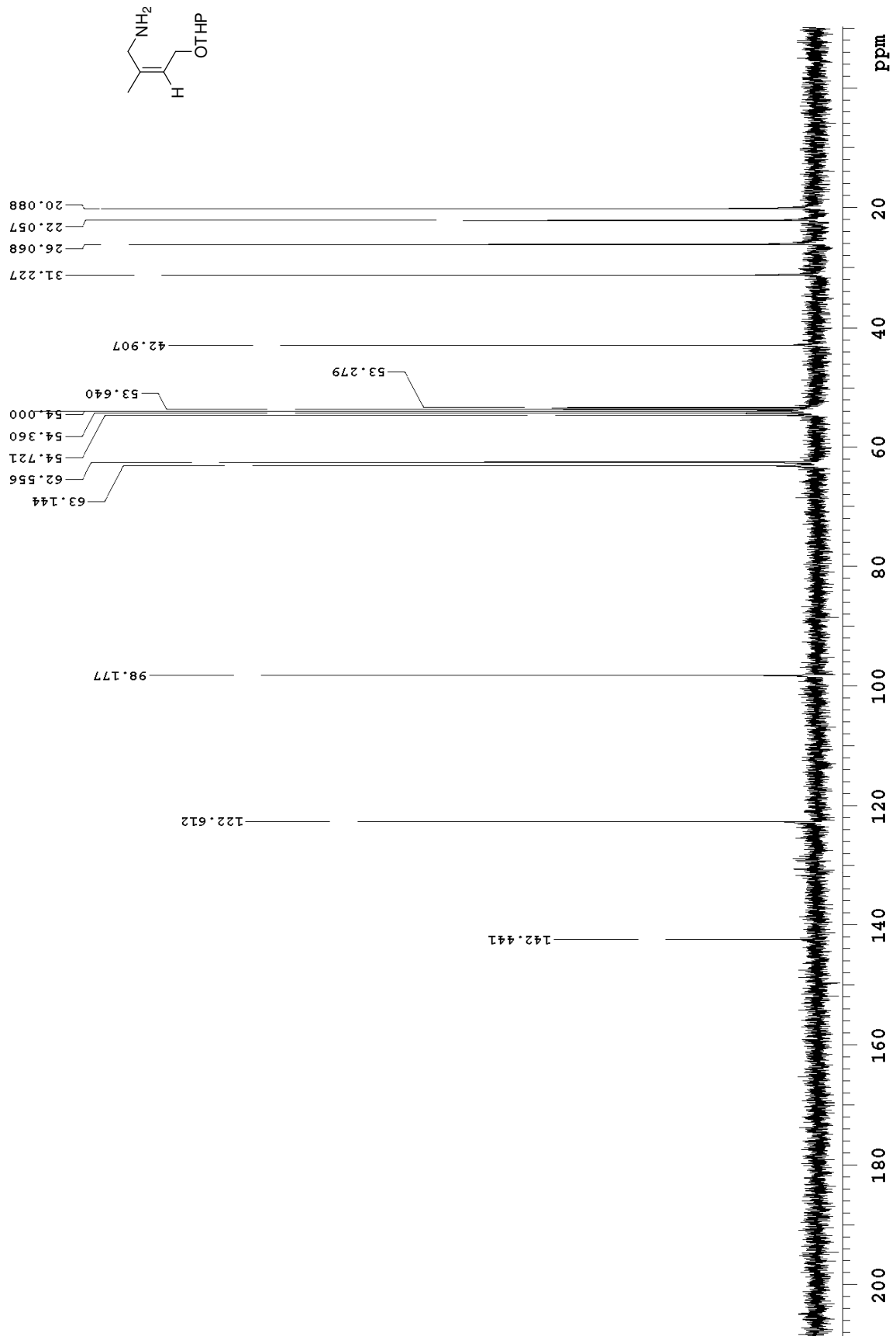

OH
OTHP
H
0.000
1.862
1.862
4.696
4.687
4.222
4.199
4.156
4.135
4.108
4.071
4.031
1.90
5.2
4.8
4.4
4.0
ppm
1.00
1.08
4.42
1.10
1.13
1.19
10.01
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
ppm

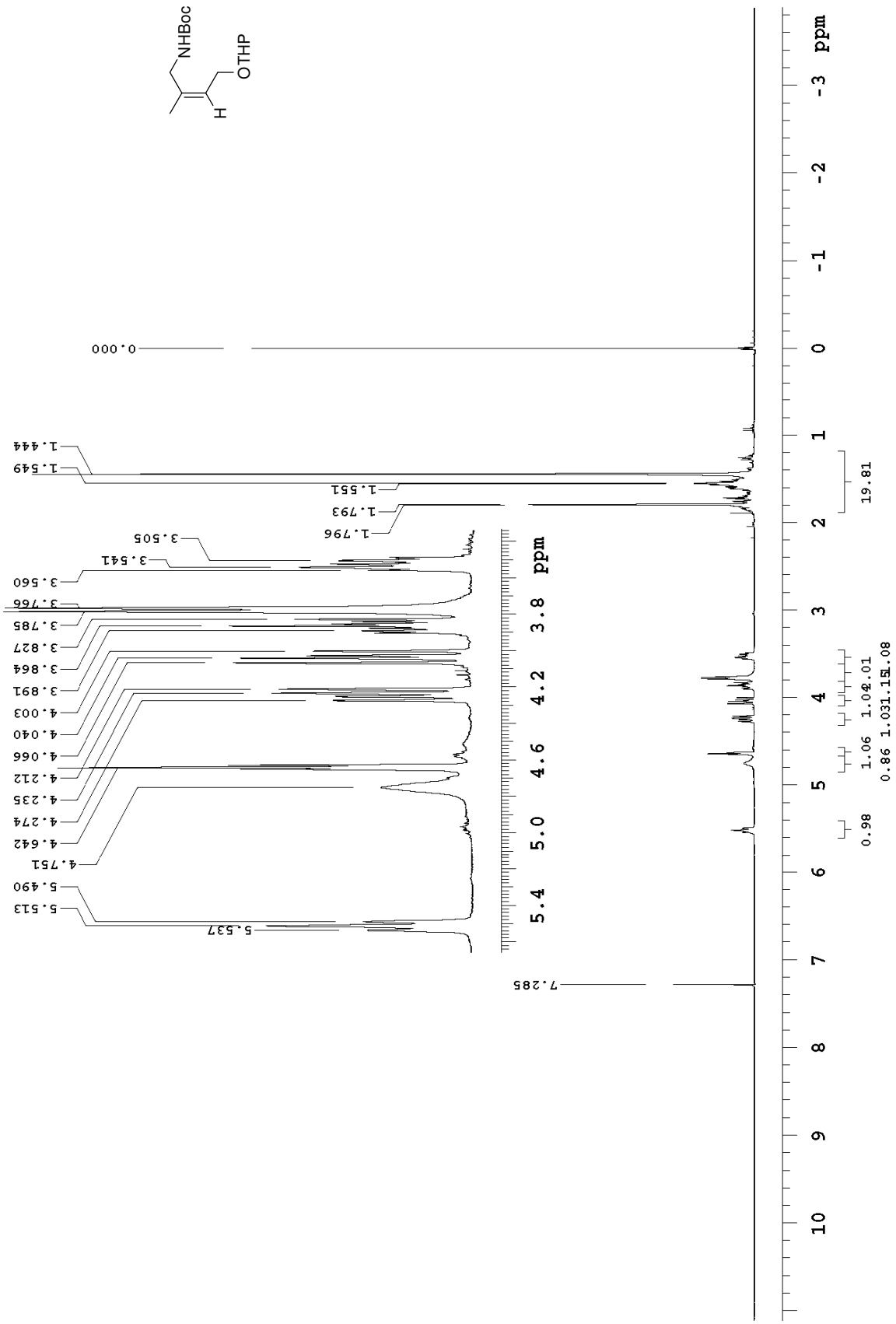

OH
OTHP
H
-0.068
19.140
21.850
25.288
30.373
61.416
61.956
62.423
76.580
77.000
77.427
96.983
123.253
141.007
0 ppm
20
40
60
80
100
120
140
160
180
200

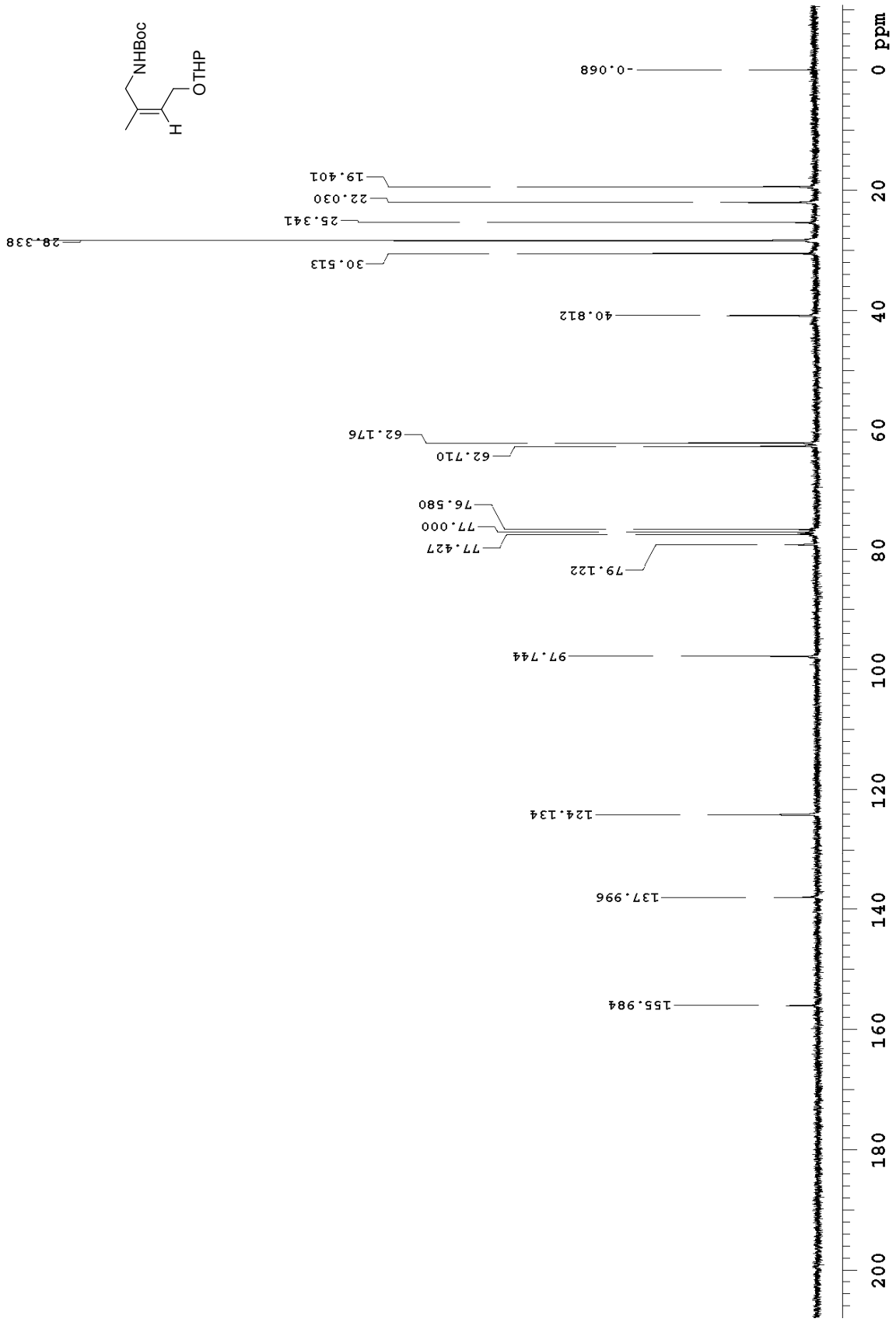

OTHP
H
N
O
O
ppm
10.40
1.12
1.12
1.1218
2.19
1.03
1.00
3.75

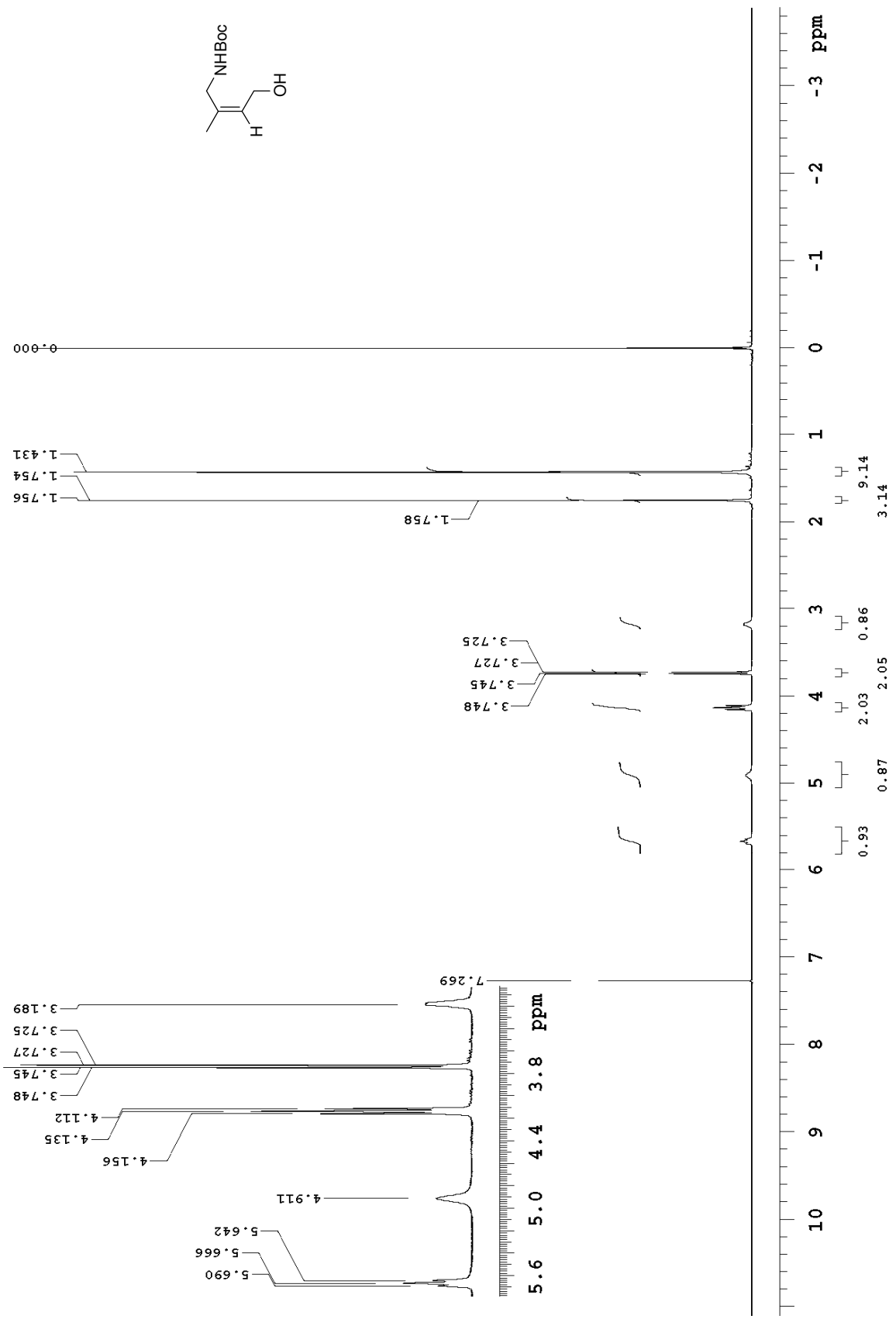

O
N
O
OTHP
H
20.128
21.850
26.095
31.254
38.763
53.279
53.640
54.000
54.360
54.721
62.650
63.511
98.551
123.626
126.703
132.616
134.298
134.512
168.664
ppm
20
40
60
80
100
120
140
160
180
200

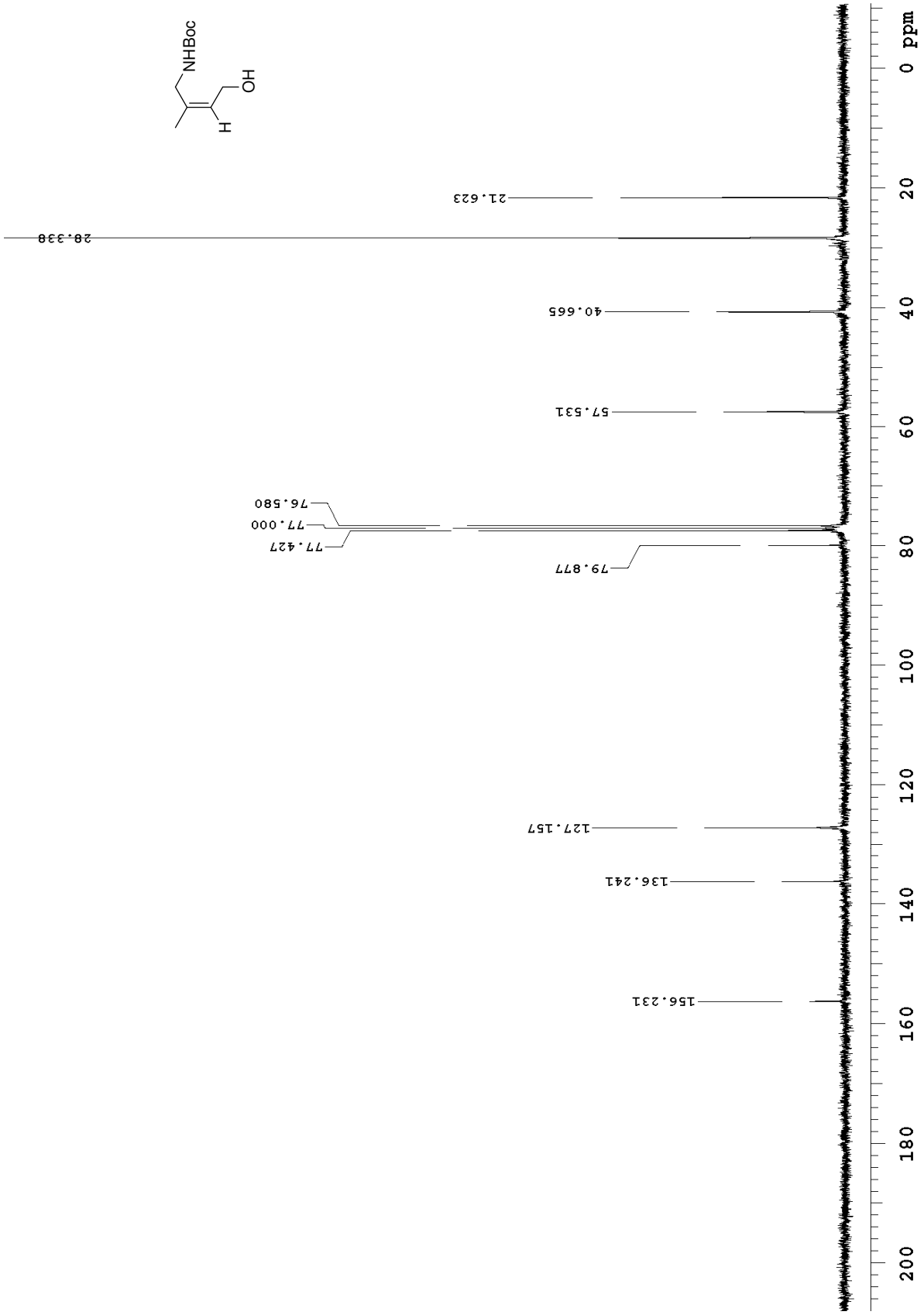

NH2
OTHP
H
1.404
1.441
1.449
1.460
1.469
1.480
1.485
1.493
1.503
1.515
1.522
1.530
1.536
1.541
1.548
1.556
1.631
1.641
1.661
1.671
1.691
1.701
1.707
1.755
1.760
1.765
1.770
1.799
3.234
3.441
3.446
3.464
3.481
3.488
3.498
3.782
3.794
3.807
3.820
3.832
3.848
3.858
3.947
3.972
3.986
4.012
4.148
4.171
4.188
4.210
4.573
4.582
4.597
5.320
5.347
5.352
5.373
5.378
5.395
5.400
ppm
14.53
1.00
1.14
1.21
1.31
1.25
1.26
2.26

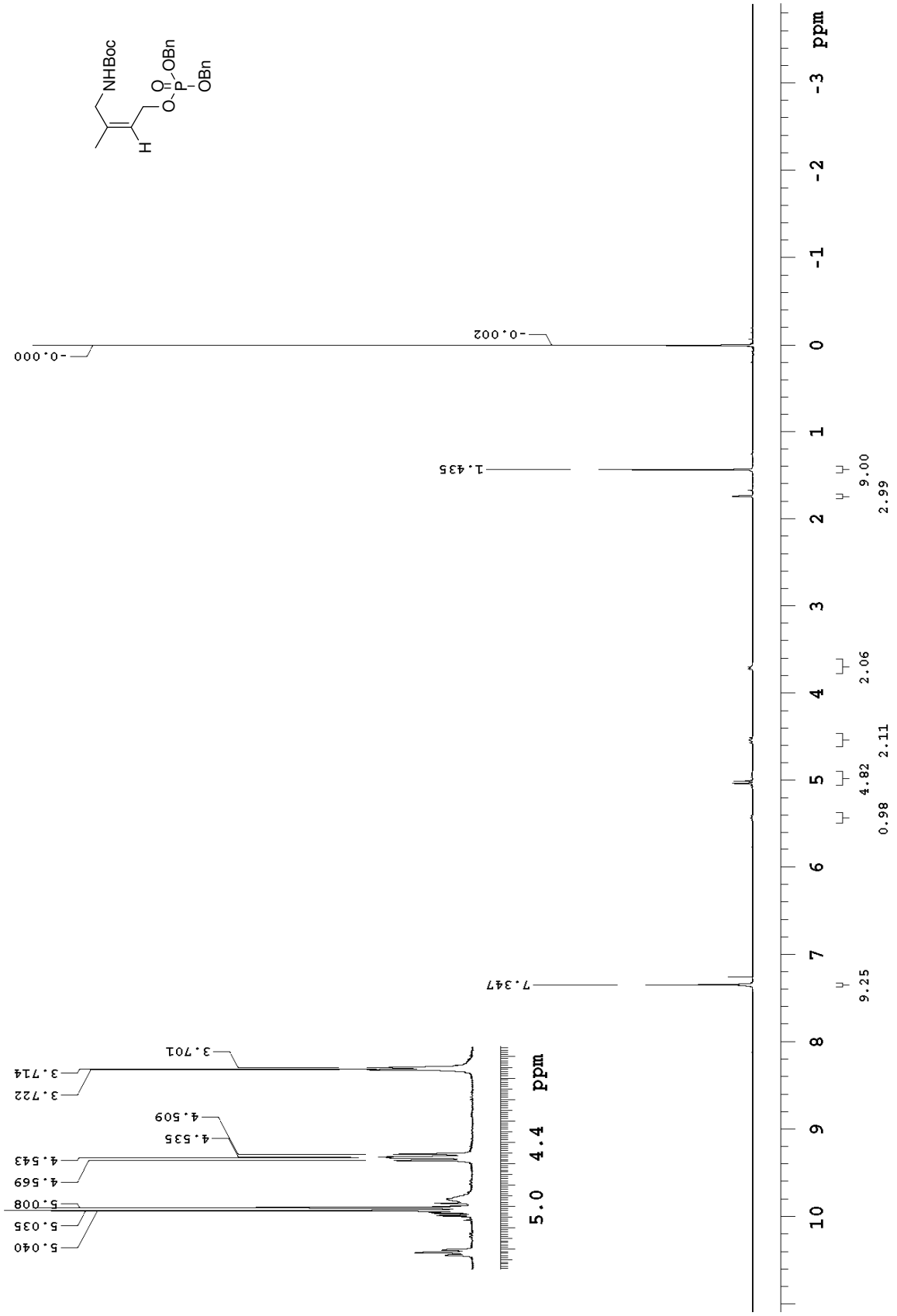

NH2
OTHP
H
ppm
20.088
22.057
26.068
31.227
42.907
53.279
53.640
54.000
54.360
54.721
62.556
63.144
98.177
122.612
142.441

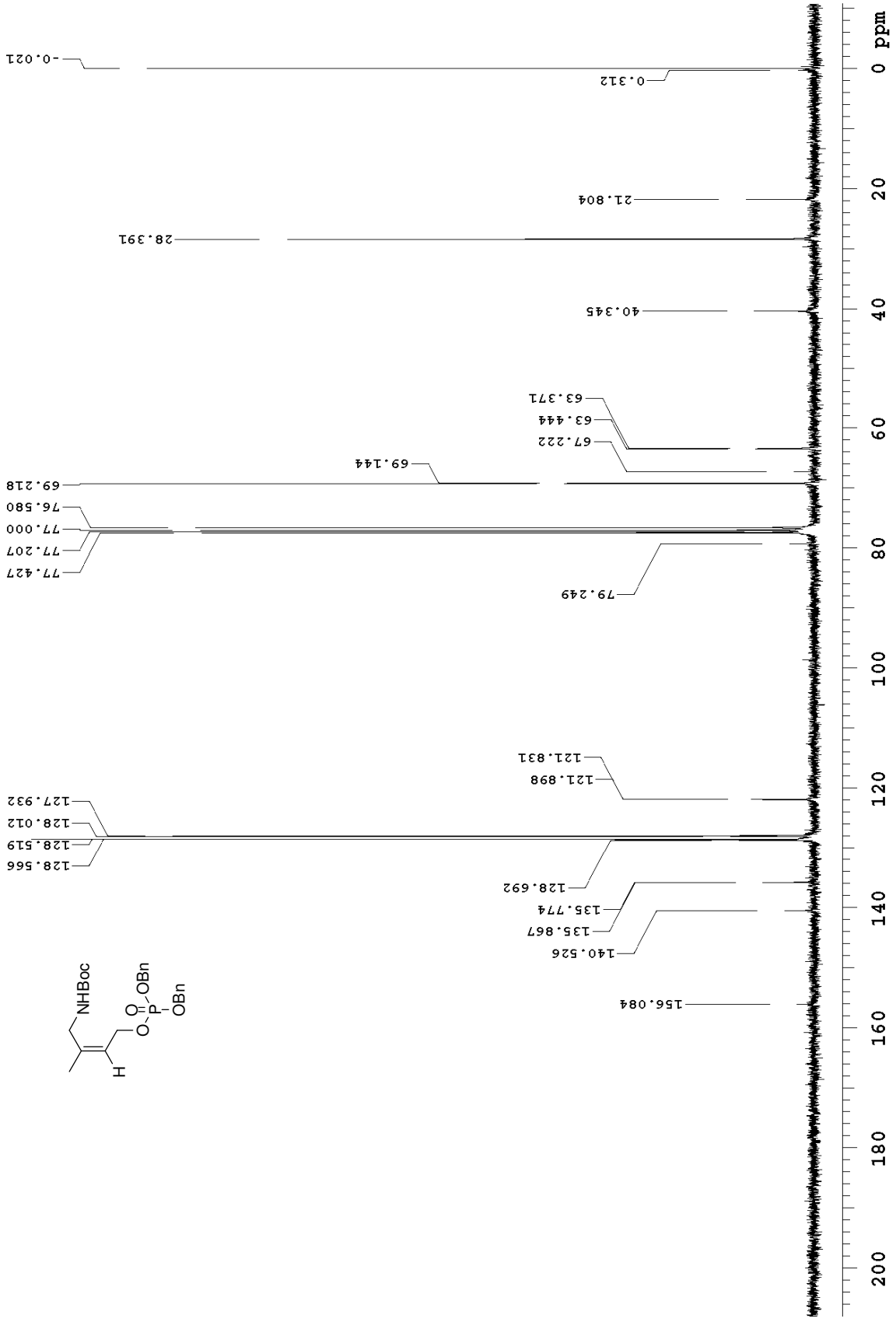

NHBoc
OTHP
H
0.000
1.444
1.549
1.551
1.793
1.796
3.505
3.541
3.560
3.766
3.785
3.827
3.864
3.891
4.003
4.040
4.066
4.212
4.235
4.274
4.642
4.751
5.490
5.513
5.537
7.285
ppm
19.81
1.06
1.04
1.01
0.98
0.86
1.03
1.15
1.08

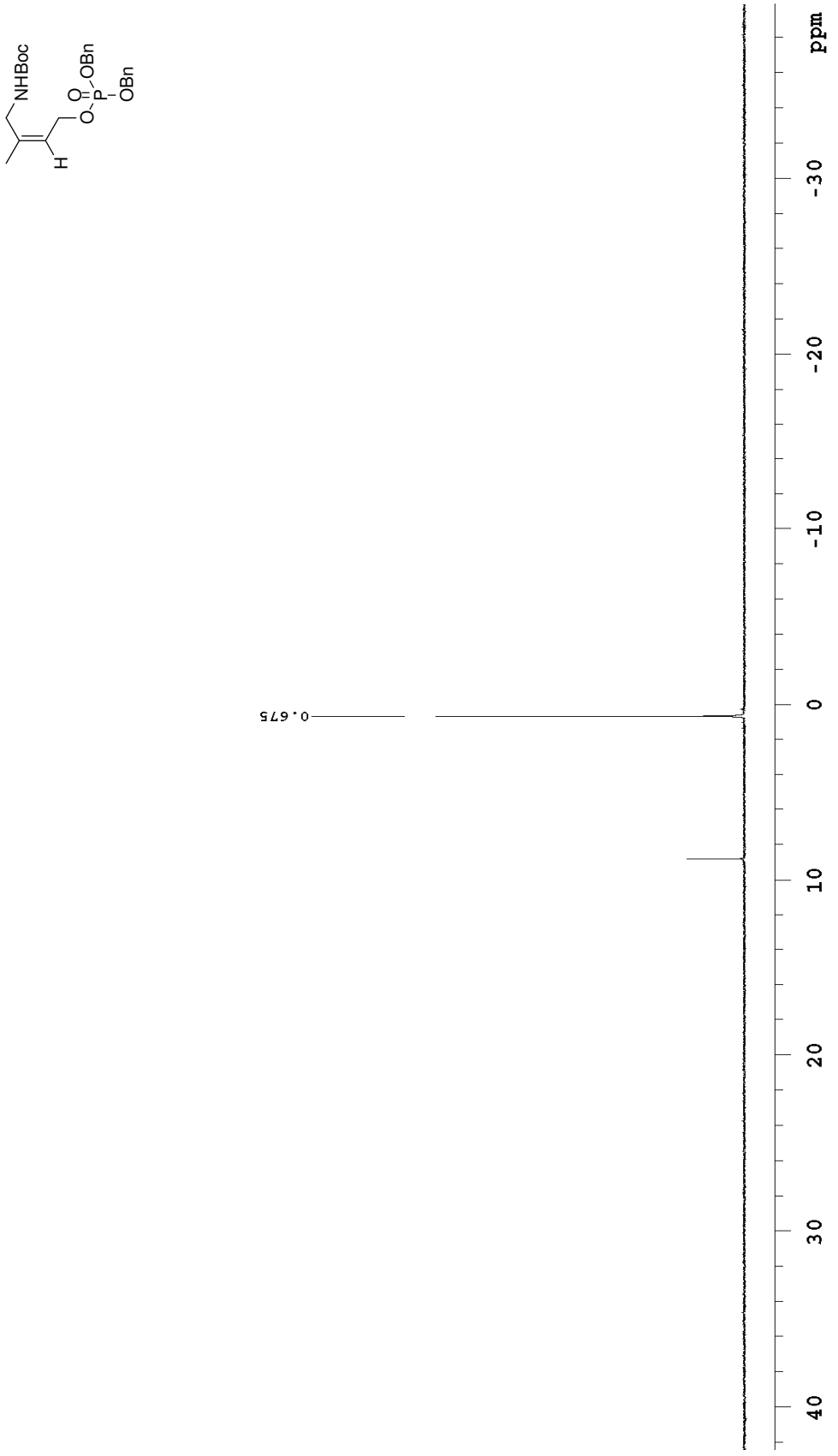

NHBoc
OTHP
H
-0.068
19.401
22.030
25.341
28.338
30.513
40.812
62.176
62.710
76.580
77.000
77.427
79.122
97.744
124.134
137.996
155.984
0 ppm
20
40
60
80
100
120
140
160
180
200

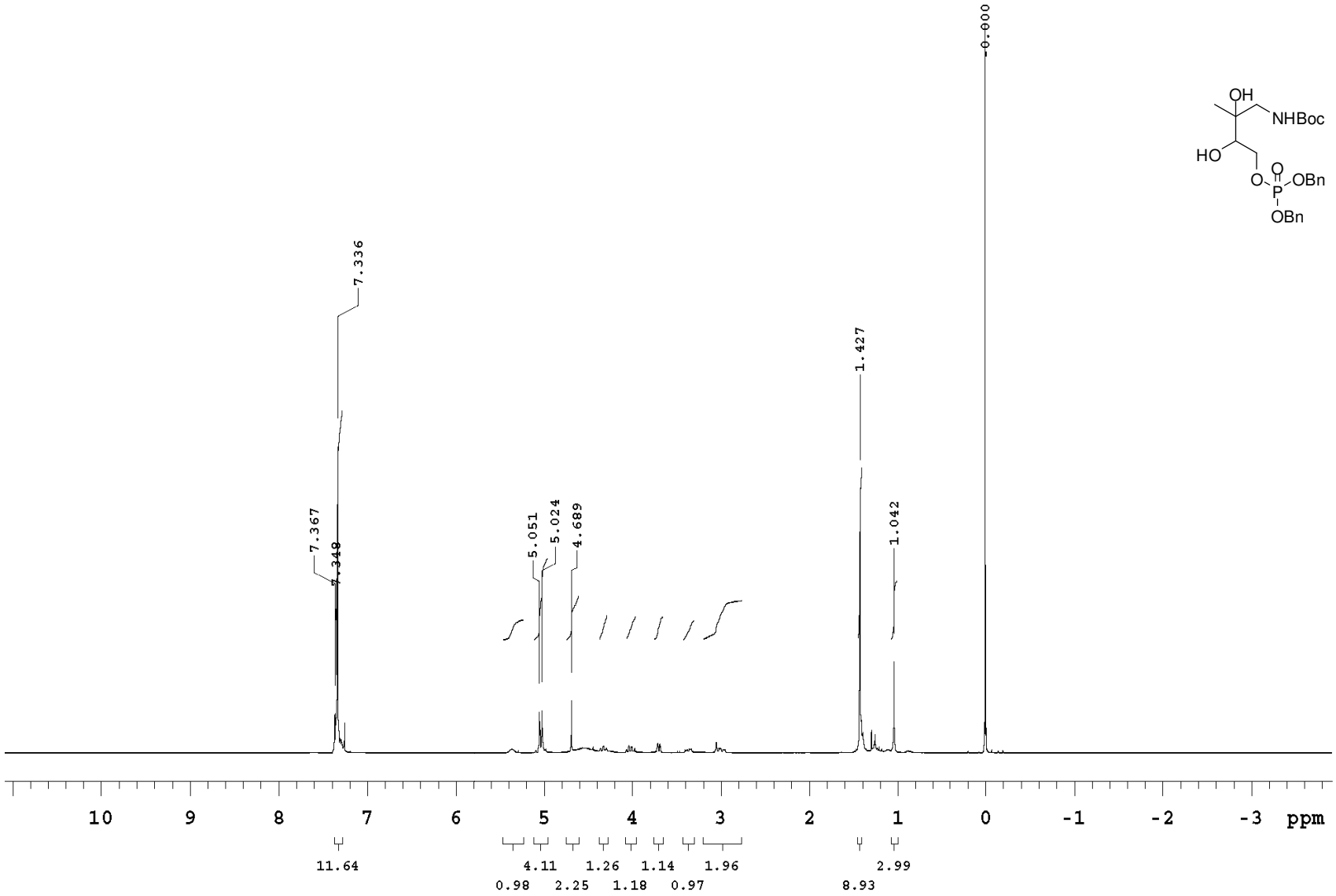

NHBoc
OH
H
0.000
1.431
1.754
1.756
1.758
3.725
3.727
3.745
3.748
4.112
4.135
4.156
4.911
5.642
5.666
5.690
7.269
3.189
ppm
0.93
0.87
2.03
0.86
2.05
9.14
3.14

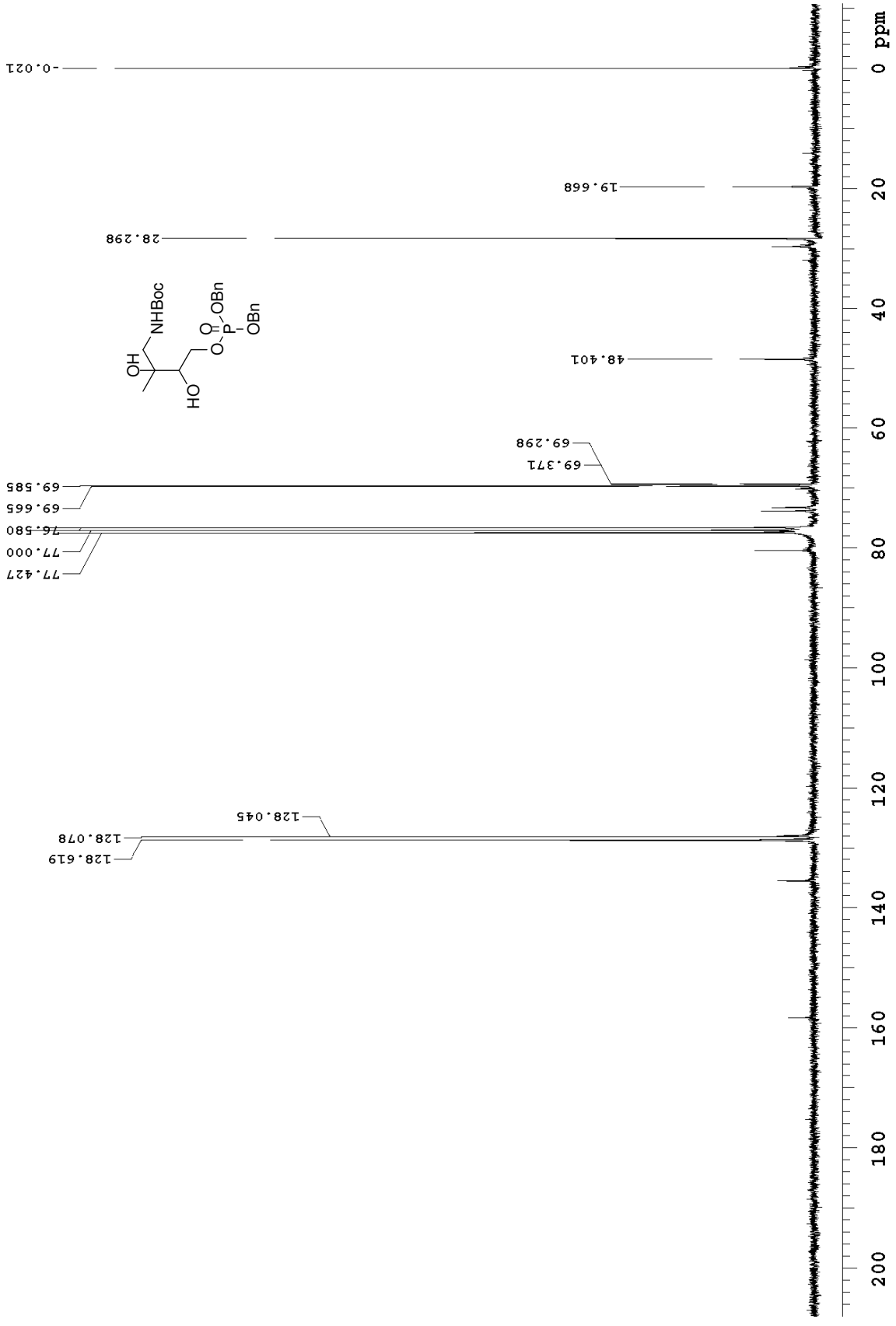

NHBoc
HO
H
156.231
136.241
127.157
79.877
77.427
77.000
76.580
57.531
40.665
28.338
21.623
0 ppm
20
40
60
80
100
120
140
160
180
200

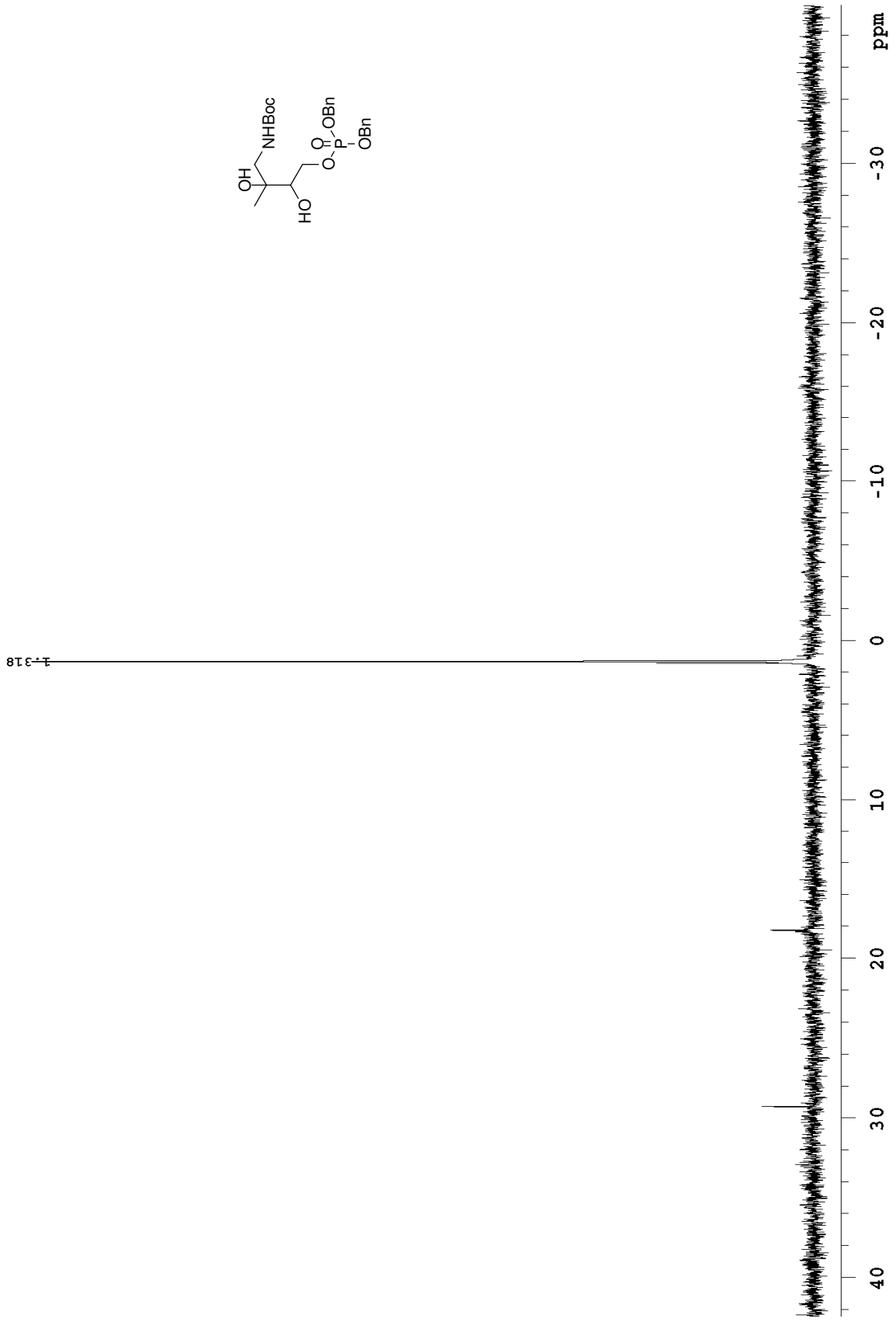

NHBoc
O
P
OBn
OBn
H
-0.000
-0.002
1.435
7.347
3.701
3.714
3.722
4.509
4.535
4.543
4.569
5.008
5.035
5.040
5.0 4.4 ppm
9.25
0.98
4.82
2.11
2.06
9.00
2.99
10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 ppm

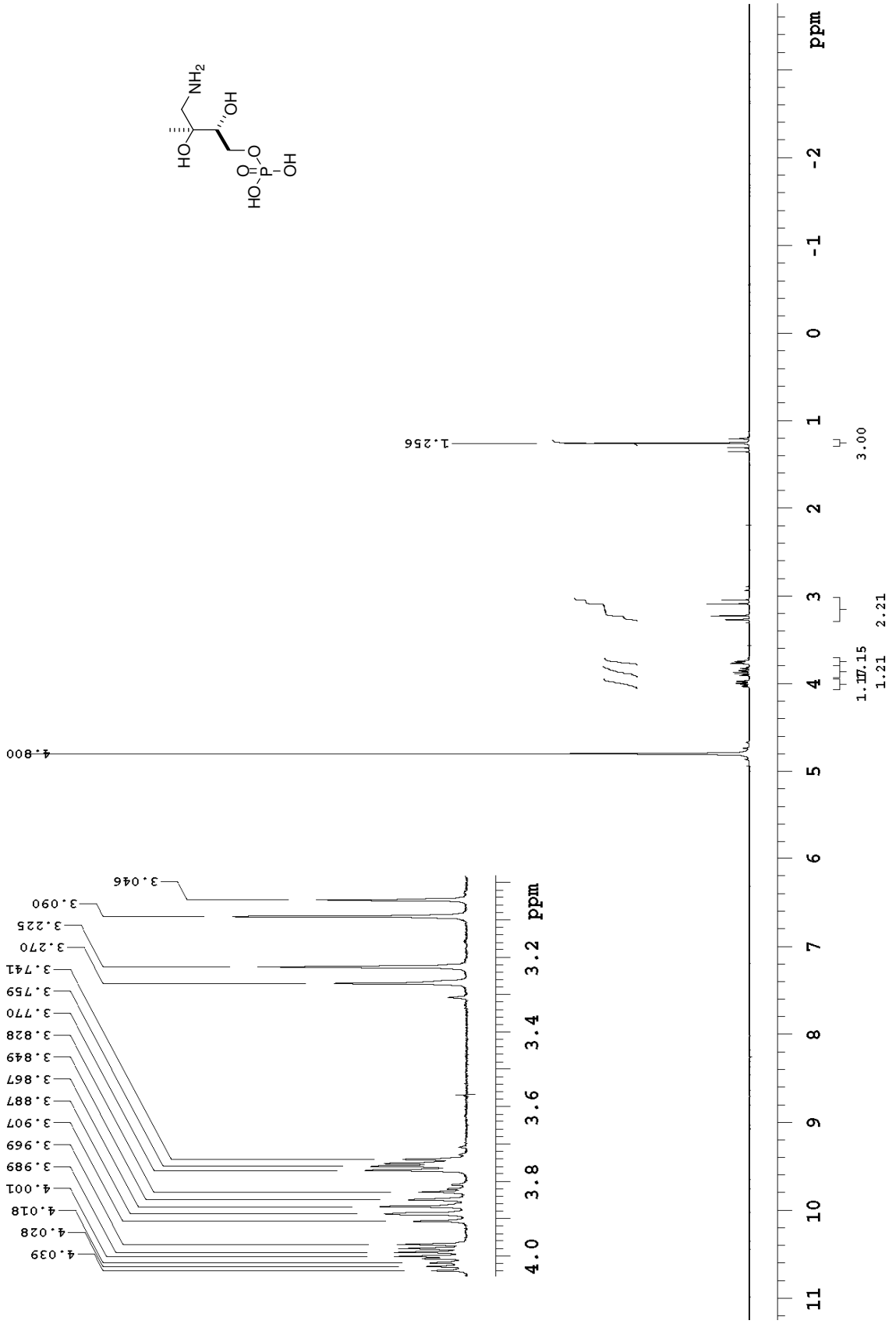

-0.021
0.312
21.804
28.391
40.345
63.371
63.444
67.222
69.144
69.218
76.580
77.000
77.207
77.427
79.249
121.831
121.898
127.932
128.012
128.519
128.566
128.692
135.774
135.867
140.526
156.084
NHBoc
O
P
OBn
OBn
H
0 ppm
20
40
60
80
100
120
140
160
180
200

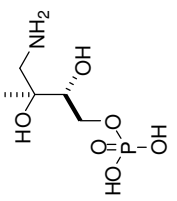

NHBoc
O
OBn
P
OBn
H
0.675
40
30
20
10
0
-10
-20
-30
ppm

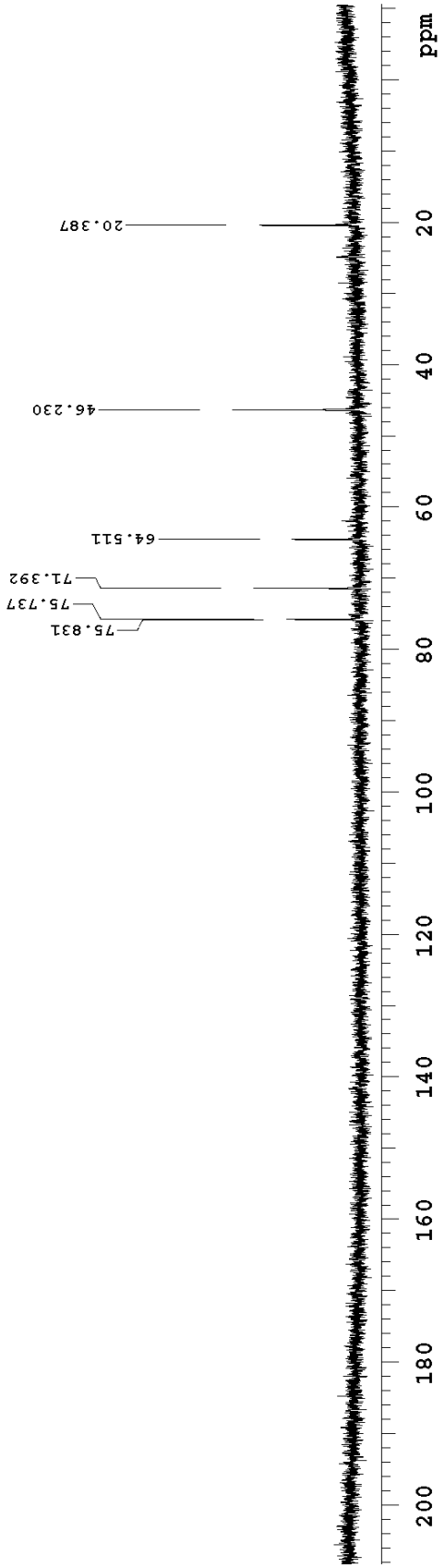

7.367
7.348
7.336
5.051
5.024
4.689
1.427
1.042
0.000
OH
NHBoc
HO
O
O
P
OBn
OBn
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
ppm
11.64
4.11
1.26
1.14
1.96
0.98
2.25
1.18
0.97
8.93
2.99

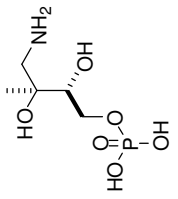

-0.021
19.668
28.298
48.401
69.298
69.371
69.585
69.665
76.580
77.000
77.427
128.045
128.078
128.619
NHBoc
OH
HO
O
P
OBn
OBn
0 ppm
20
40
60
80
100
120
140
160
180
200

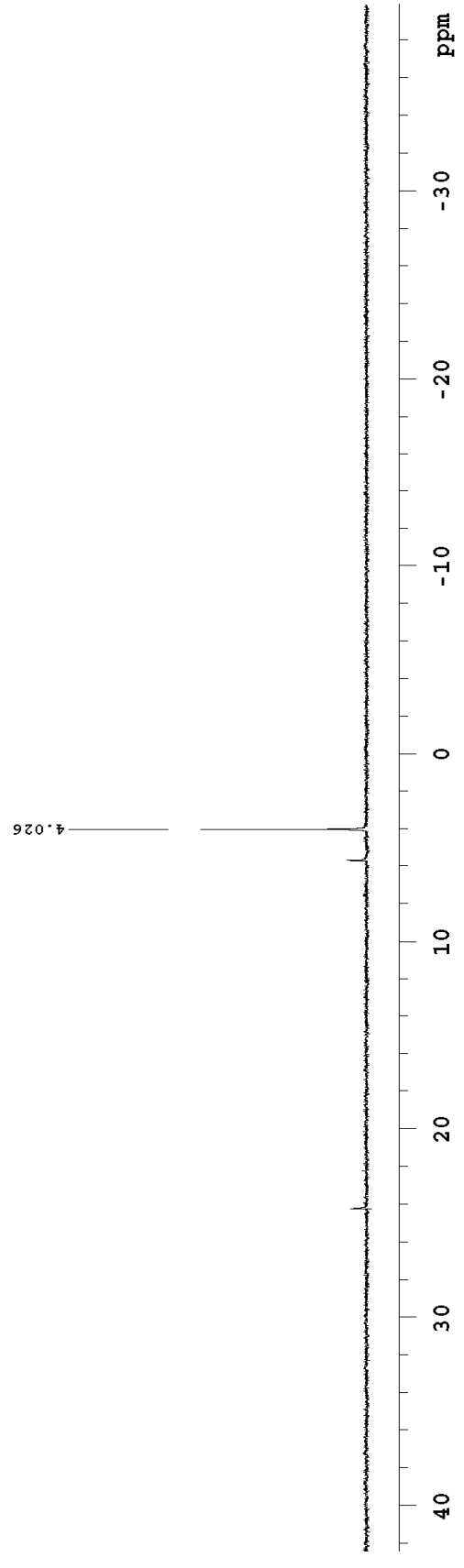

NHBoc
OH
HO
O
P
OBn
OBn
1.318
40
30
20
10
0
-10
-20
-30
ppm

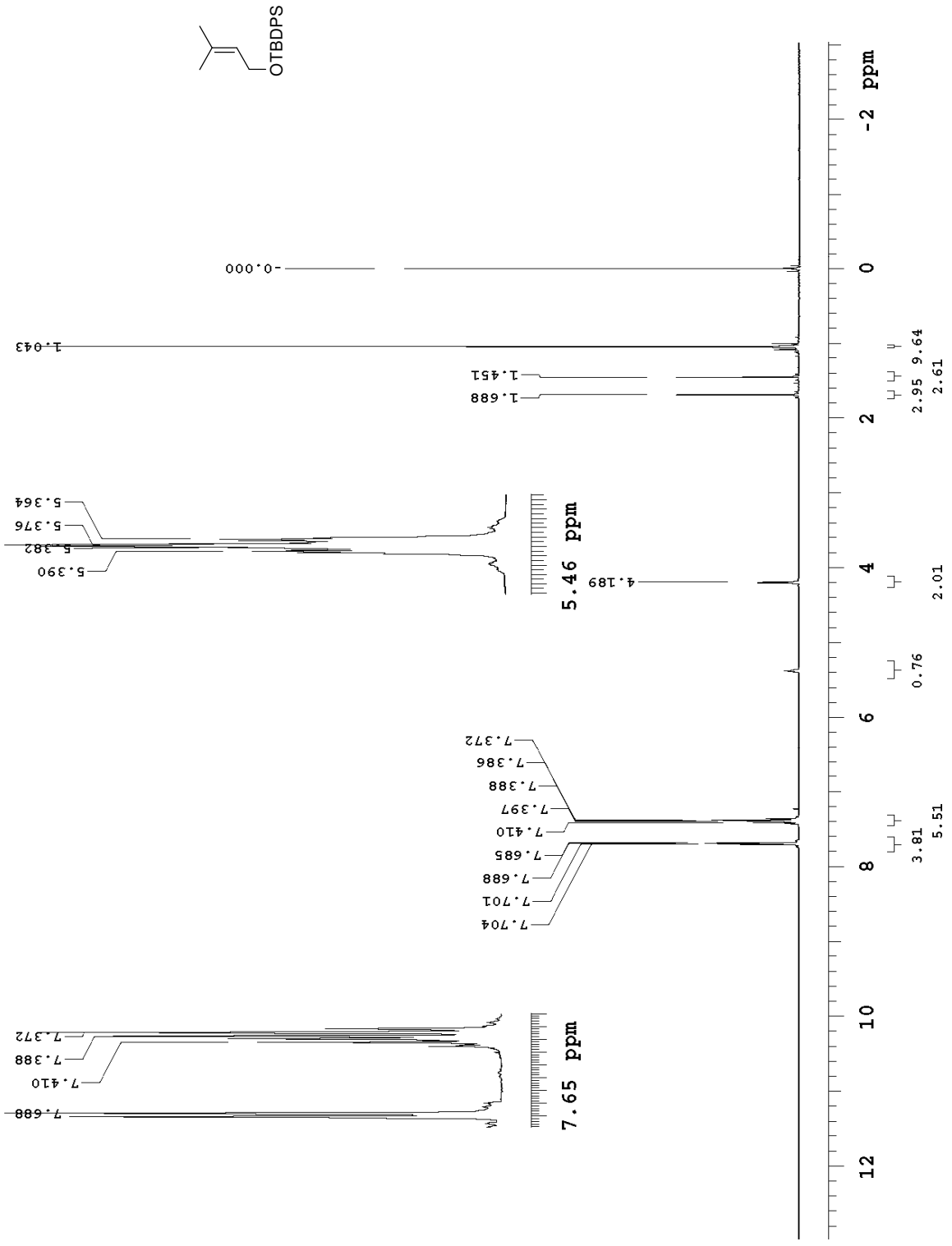

4.800
1.256
3.046
3.090
3.225
3.270
3.741
3.759
3.770
3.828
3.849
3.867
3.887
3.907
3.969
3.989
4.001
4.018
4.028
4.039
1.00
1.11
1.21
3.00
2.21
ppm
4.0
3.8
3.6
3.4
3.2
HO
NH2
OH
O
P
HO
OH

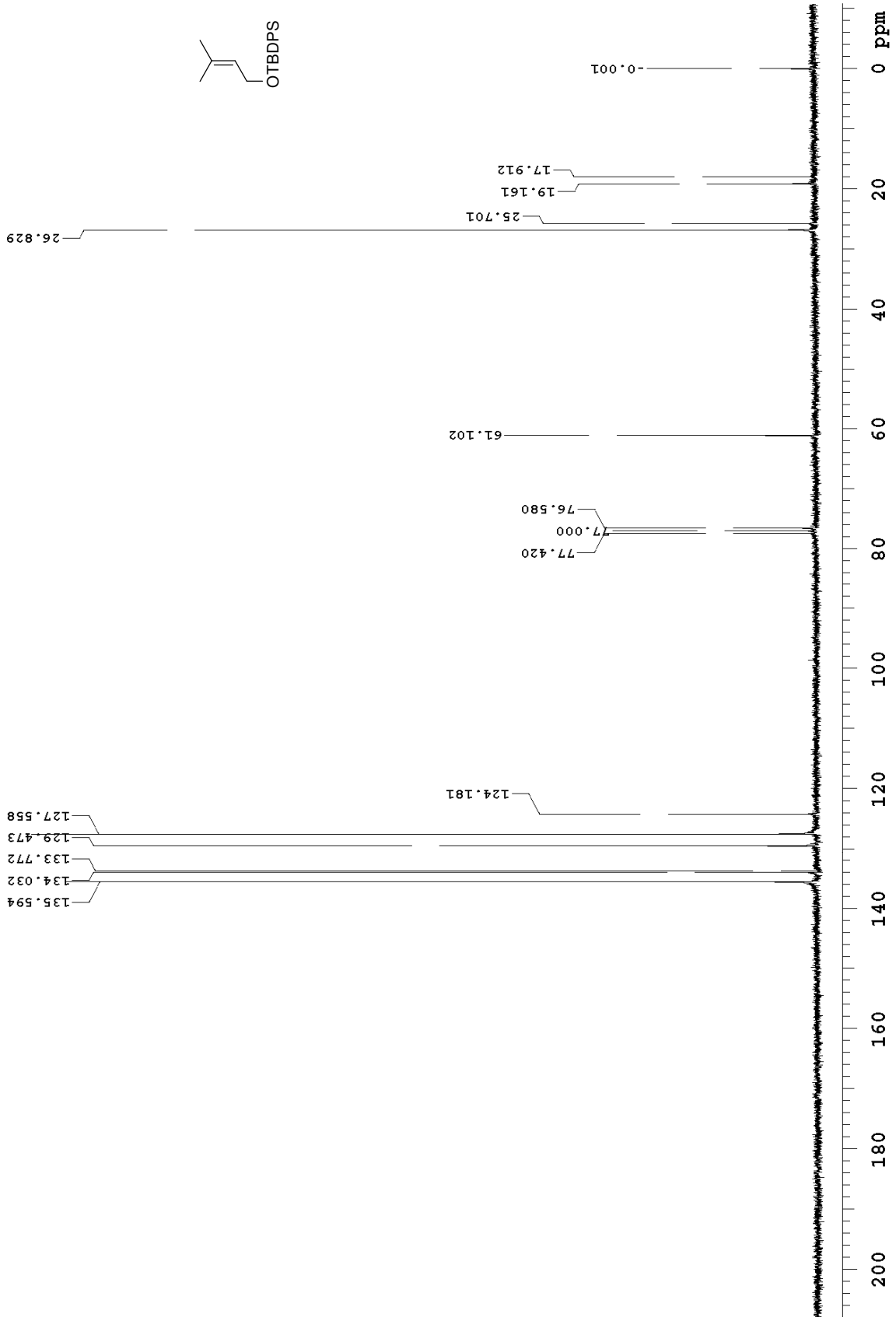

NH2
OH
HO
O
P
HO
OH
O

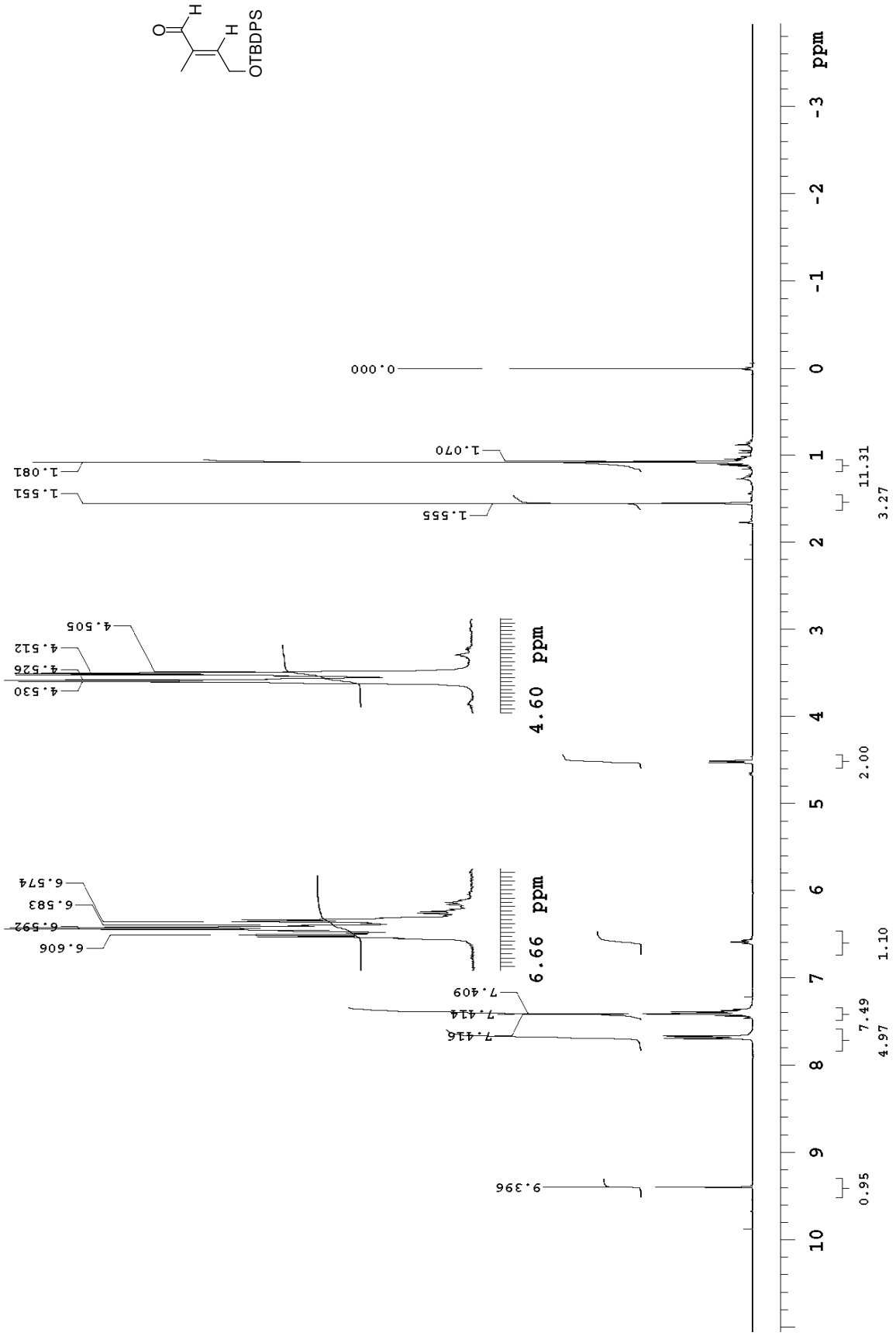

20.387
46.230
64.511
71.392
75.737
75.831
ppm
20
40
60
80
100
120
140
160
180
200

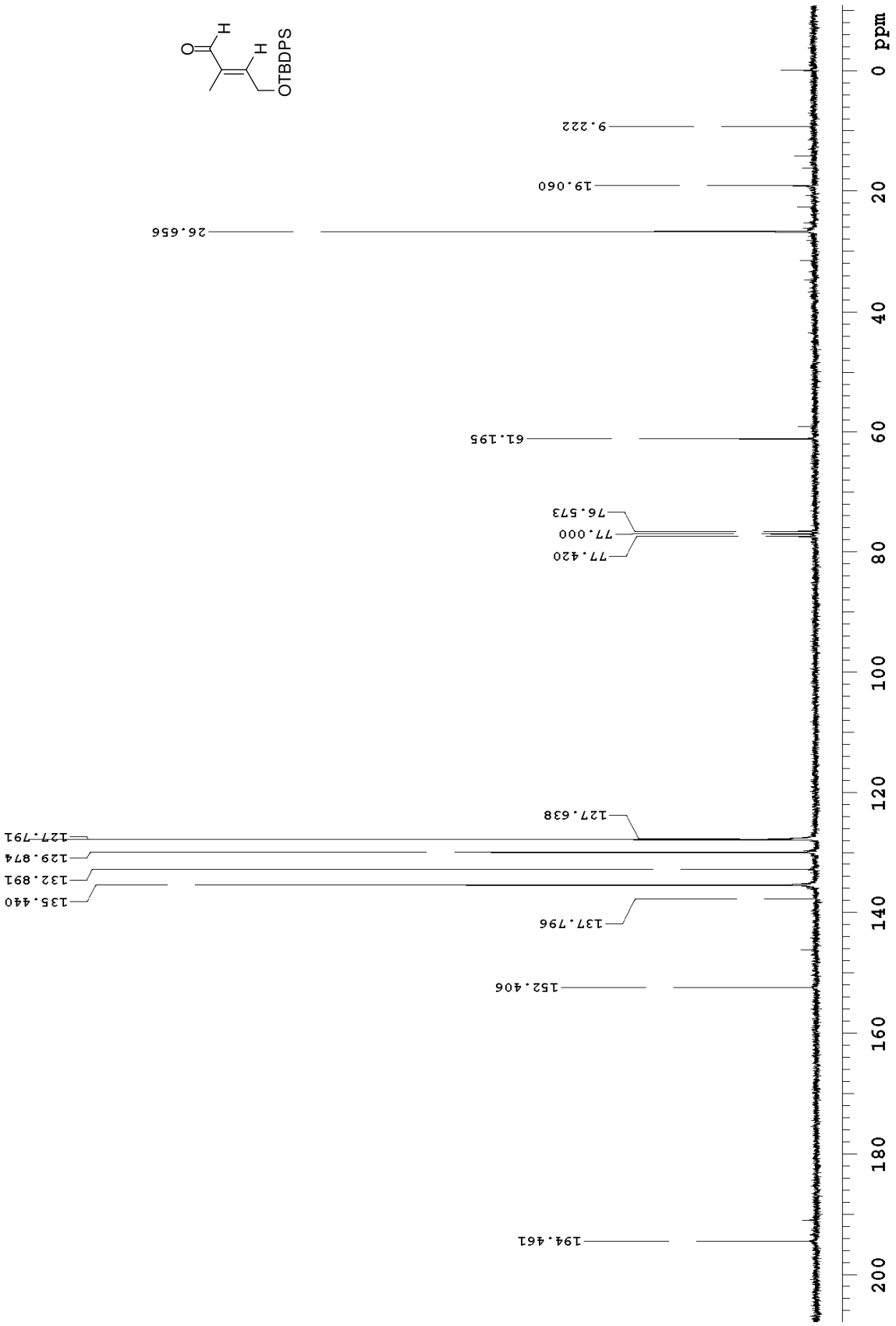

NH2
OH
HO
O
P
HO
OH

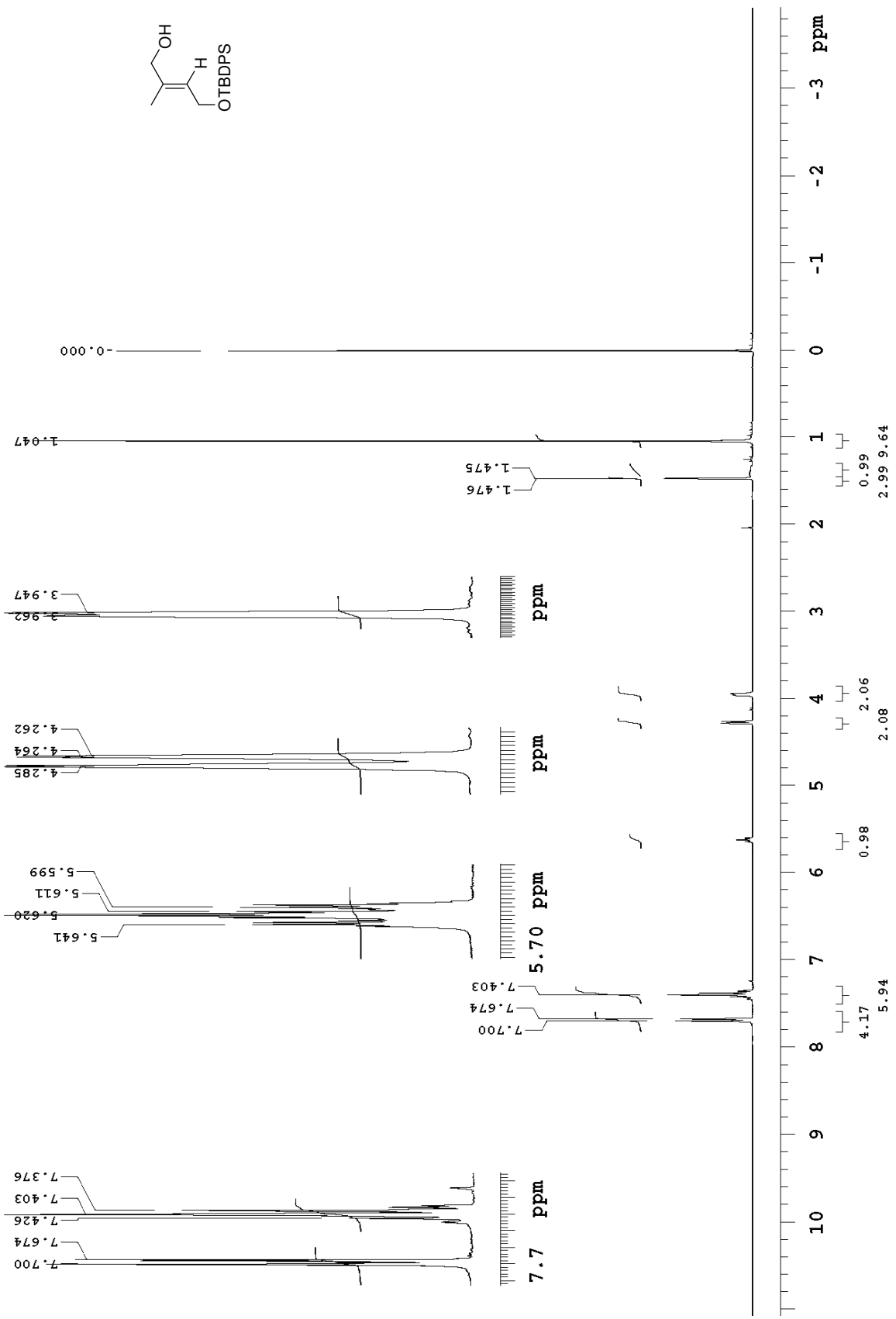

4.026
40
30
20
10
0
-10
-20
-30
ppm

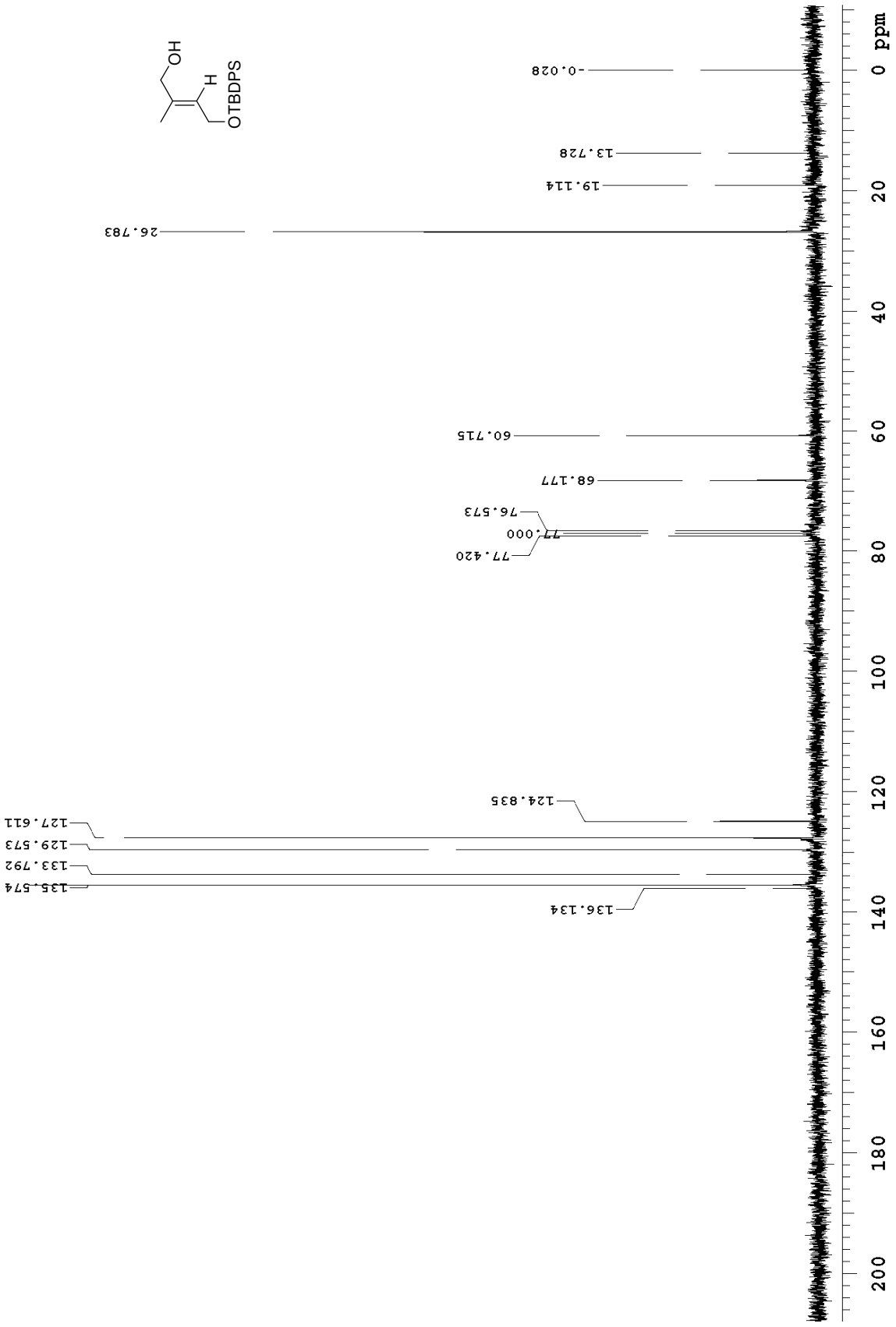

OTBDPS
-0.000
1.043
1.451
1.688
4.189
5.364
5.376
5.382
5.390
7.372
7.386
7.388
7.397
7.410
7.685
7.688
7.701
7.704
7.372
7.388
7.410
7.688
5.46 ppm
7.65 ppm
2.95
9.64
2.61
2.01
0.76
3.81
5.51
12
10
8
6
4
2
0
-2 ppm

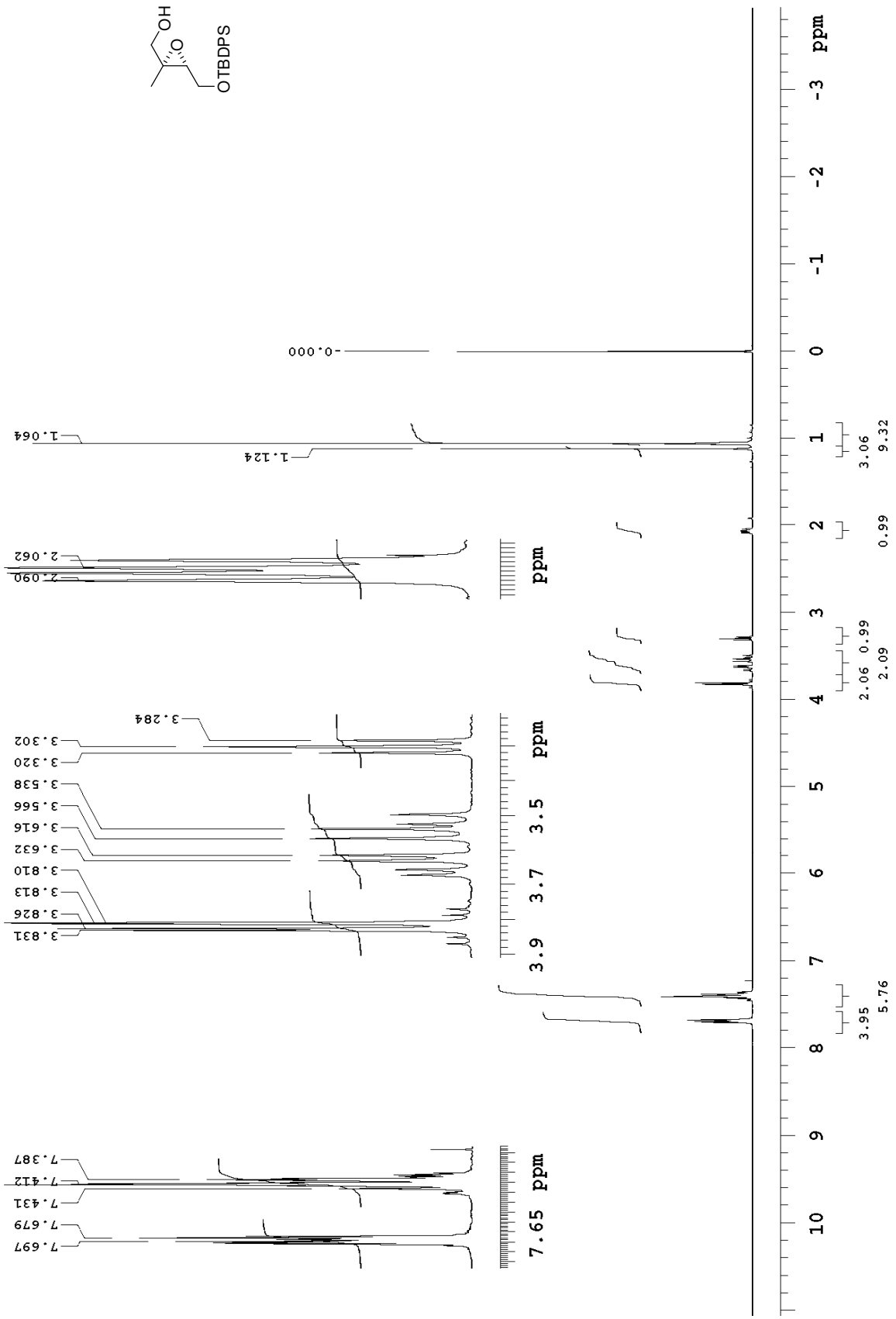

OTBDPS
-0.001
17.912
19.161
25.701
26.829
61.102
76.580
77.000
77.420
124.181
127.558
129.473
133.772
134.032
135.594
0 ppm
20
40
60
80
100
120
140
160
180
200

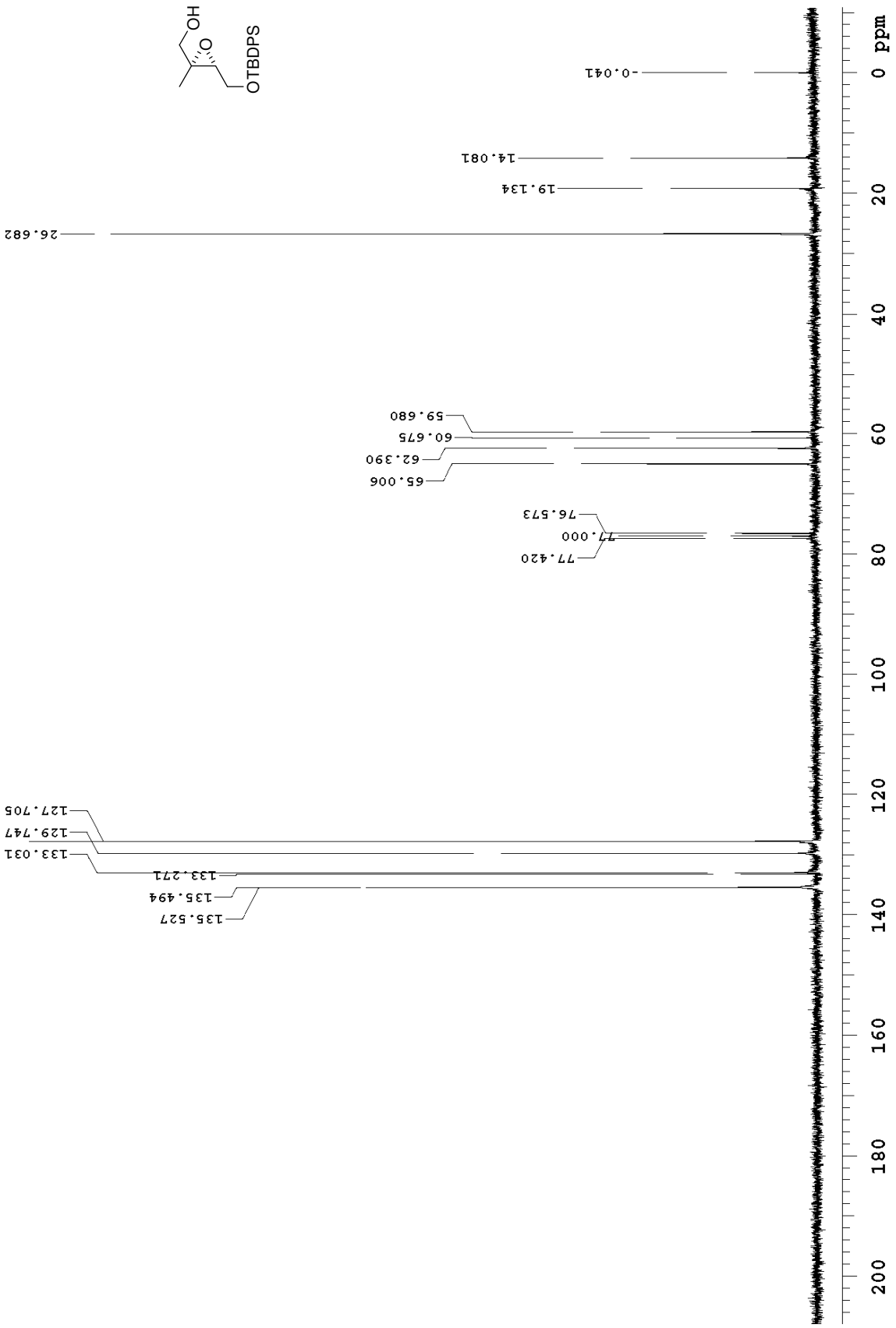

OTBDPS
9.396
7.416
7.414
7.409
6.606
6.592
6.583
6.574
4.530
4.526
4.512
4.505
1.555
1.551
1.081
1.070
0.000
6.66 ppm
4.60 ppm
ppm
0.95
7.49
4.97
1.10
2.00
11.31
3.27

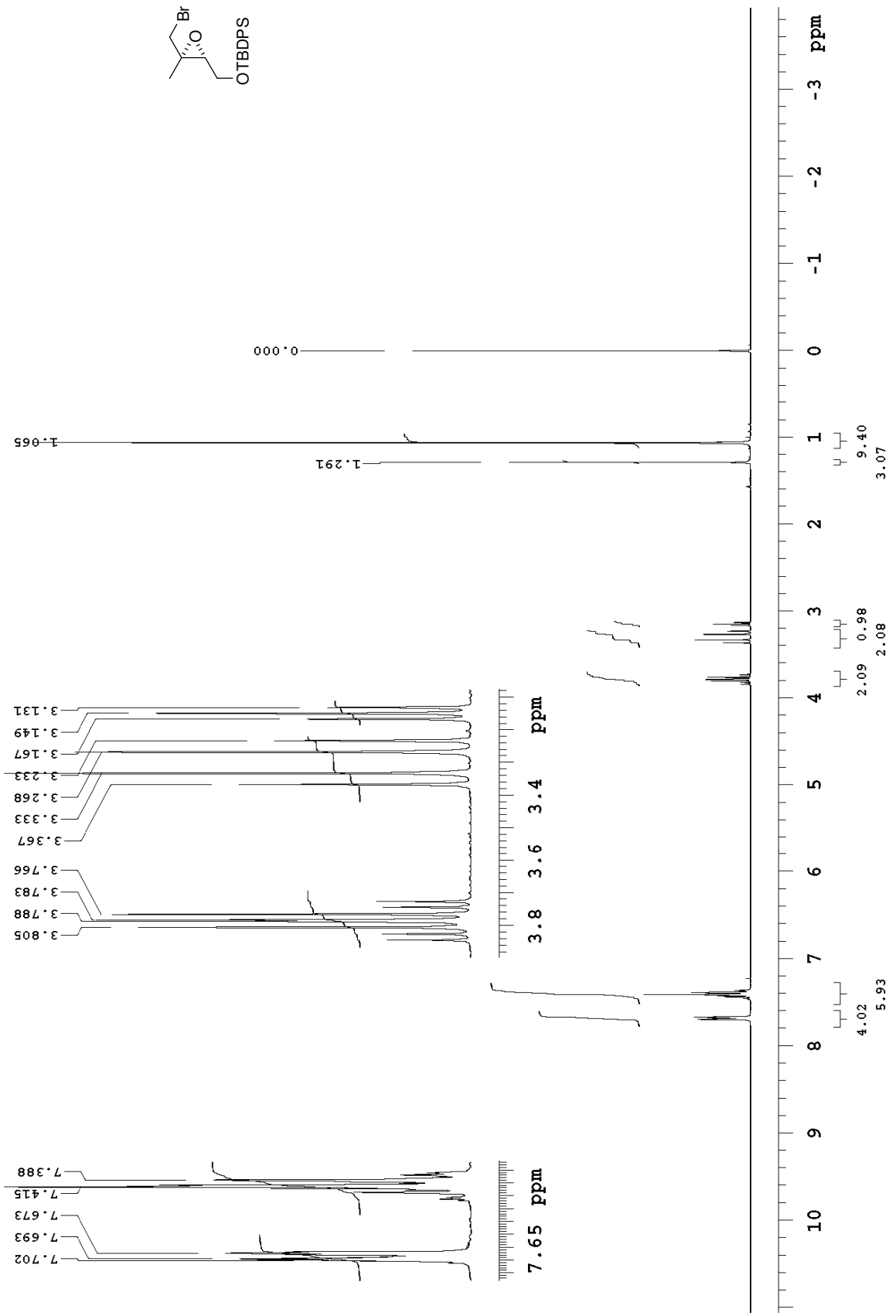

194.461
152.406
137.796
135.440
132.891
129.874
127.791
127.638
77.420
77.000
76.573
61.195
26.656
19.060
9.222
OTBDPS
0 ppm
20
40
60
80
100
120
140
160
180
200

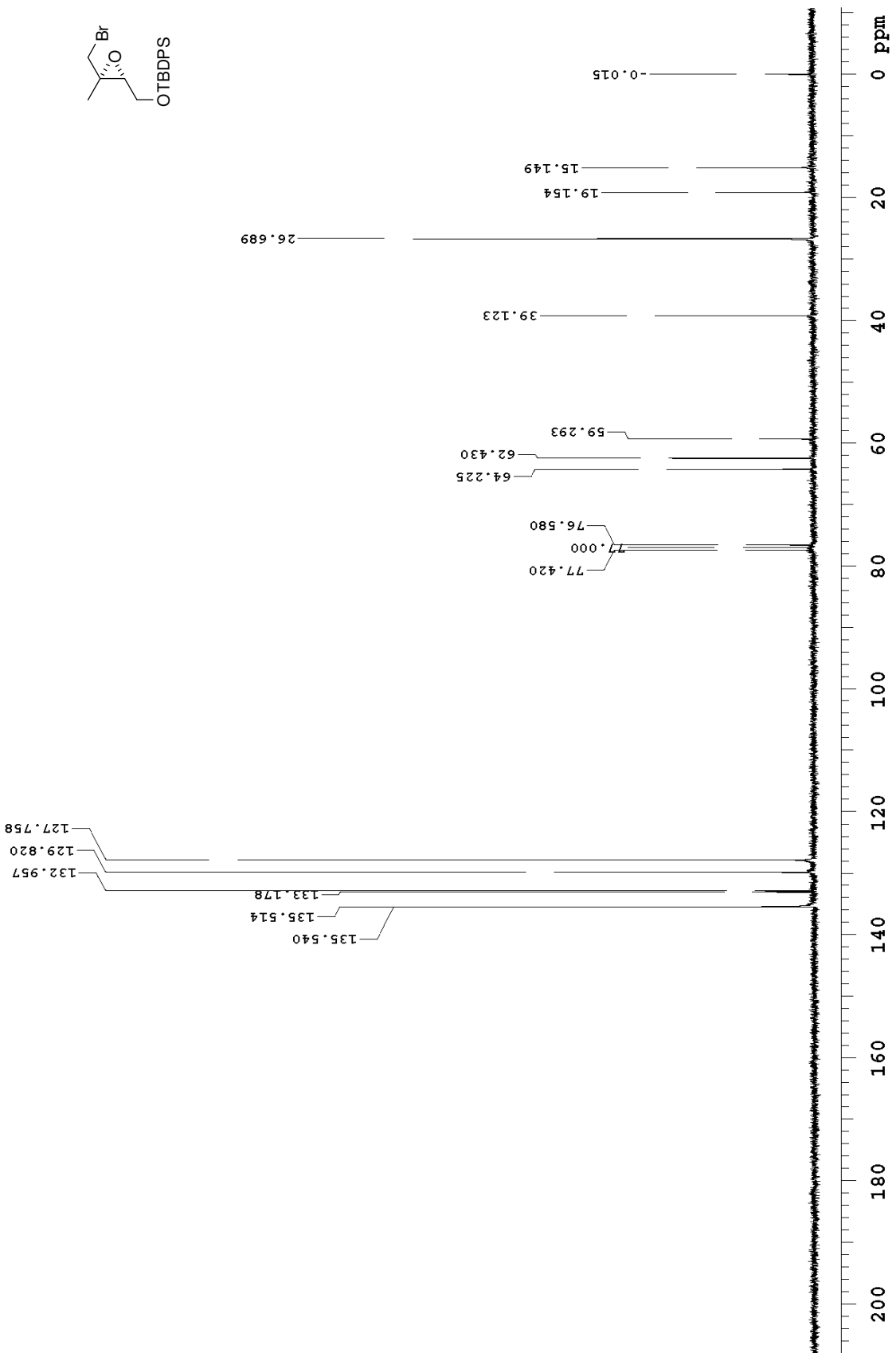

OH
H
OTBDPS
-0.000
1.047
1.476
1.475
3.947
3.962
4.285
4.264
4.262
5.641
5.620
5.611
5.599
7.700
7.674
7.403
7.426
7.403
7.376
ppm
0.99
2.99
9.64
2.06
2.08
0.98
4.17
5.94

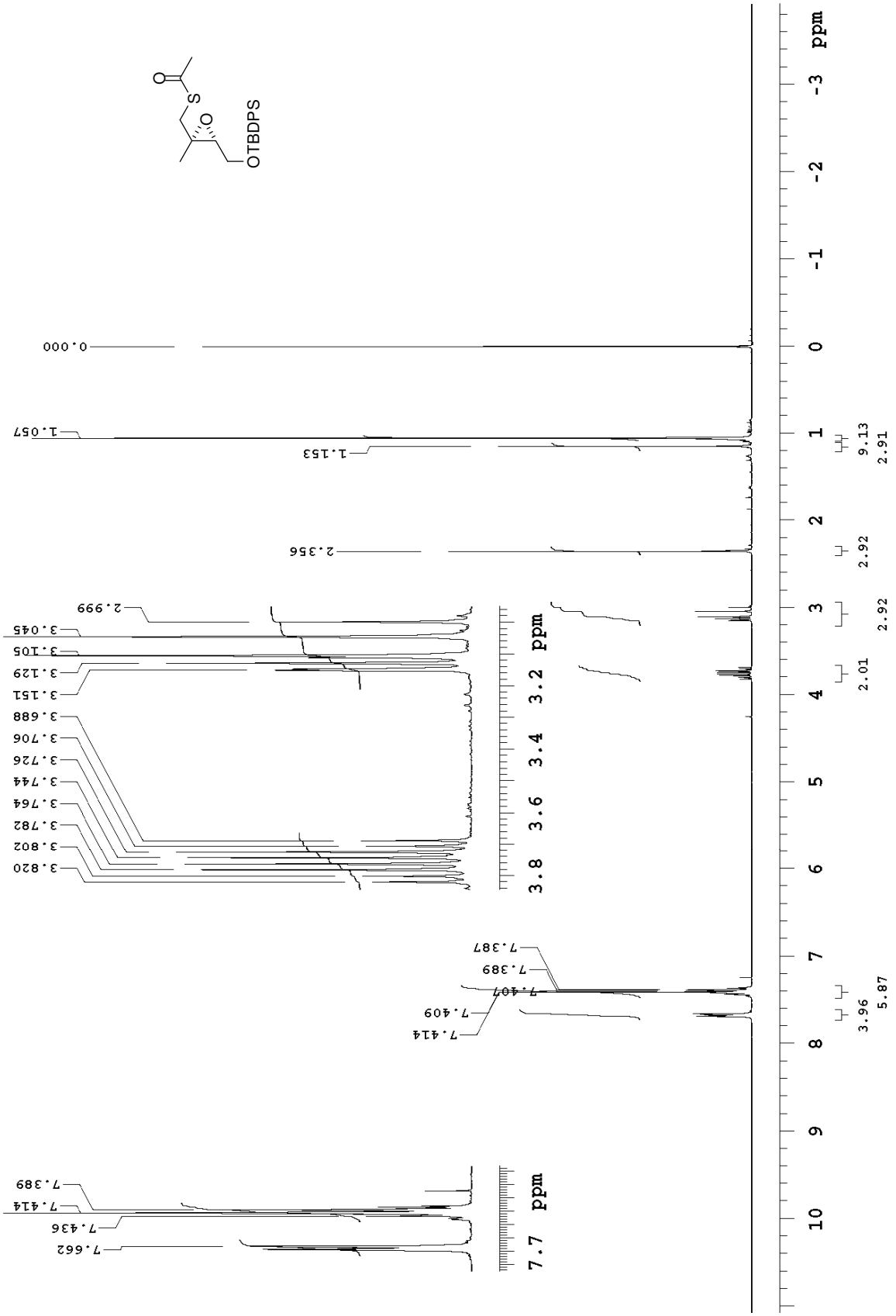

OH
H
OTBDPS
-0.028
13.728
19.114
26.783
60.715
68.177
76.573
77.000
77.420
124.835
127.611
129.573
133.792
135.574
136.134
0 ppm
20
40
60
80
100
120
140
160
180
200

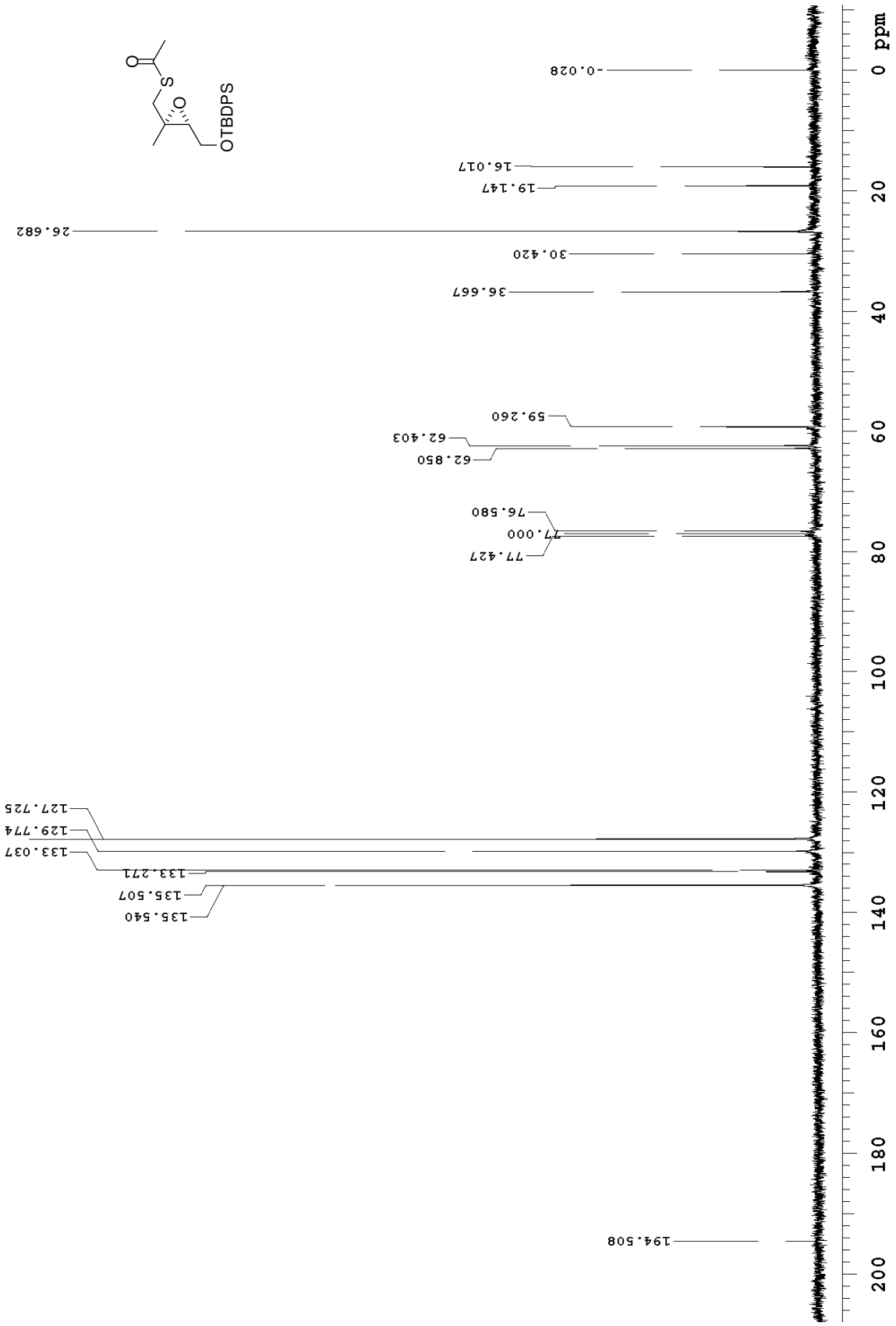

OH
O
OTBDPS
7.697
7.679
7.431
7.412
7.387
7.65 ppm
3.831
3.826
3.813
3.810
3.632
3.616
3.566
3.538
3.320
3.302
3.284
3.9 3.7 3.5 ppm
2.062
2.090
ppm
1.064
1.124
-0.000
ppm
3.95
5.76
2.06 0.99
2.09
0.99
3.06
9.32

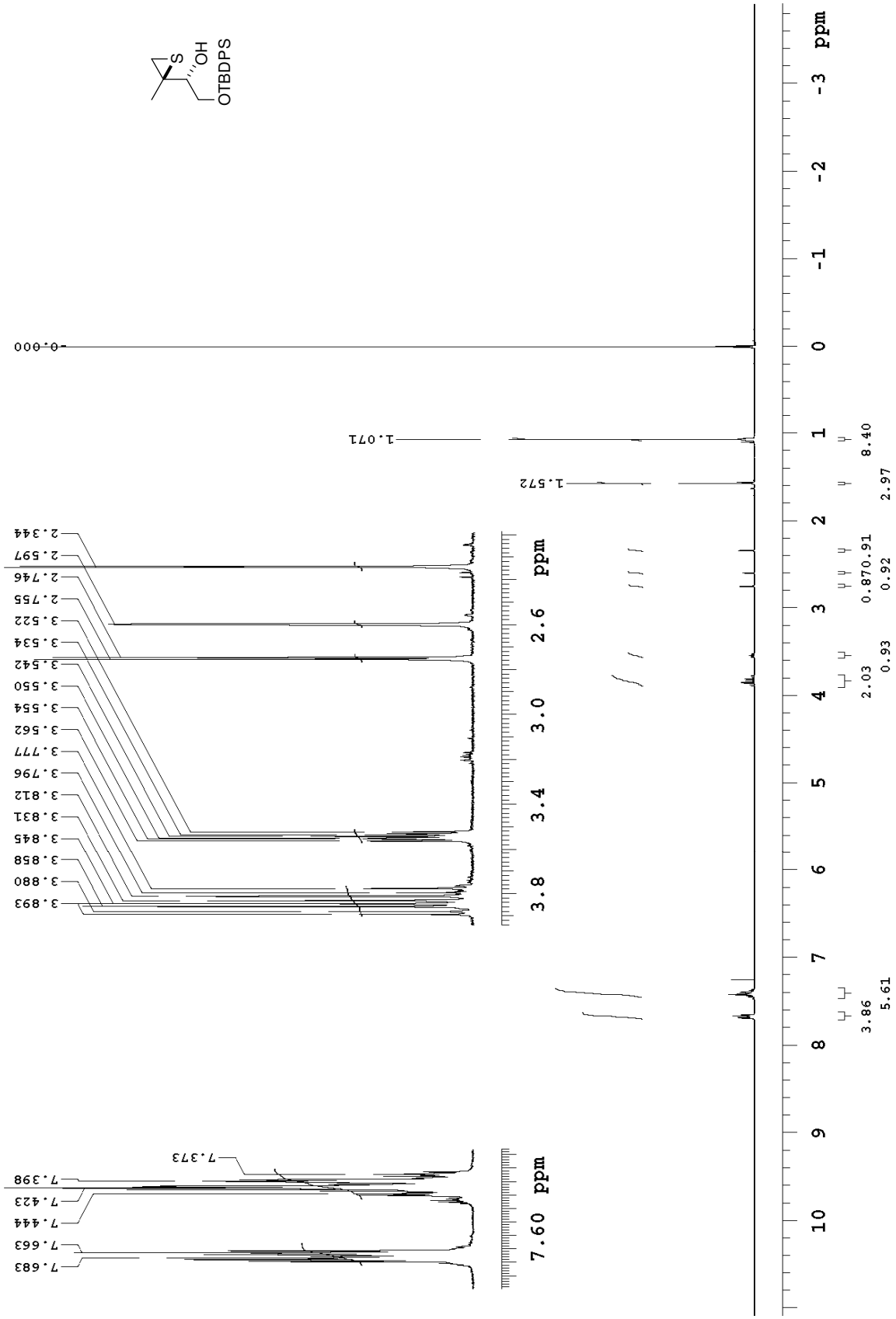

OH
O
OTBDPS
-0.041
14.081
19.134
26.682
59.680
60.675
62.390
65.006
76.573
77.000
77.420
127.705
129.747
133.031
133.271
135.494
135.527
0 ppm
20
40
60
80
100
120
140
160
180
200

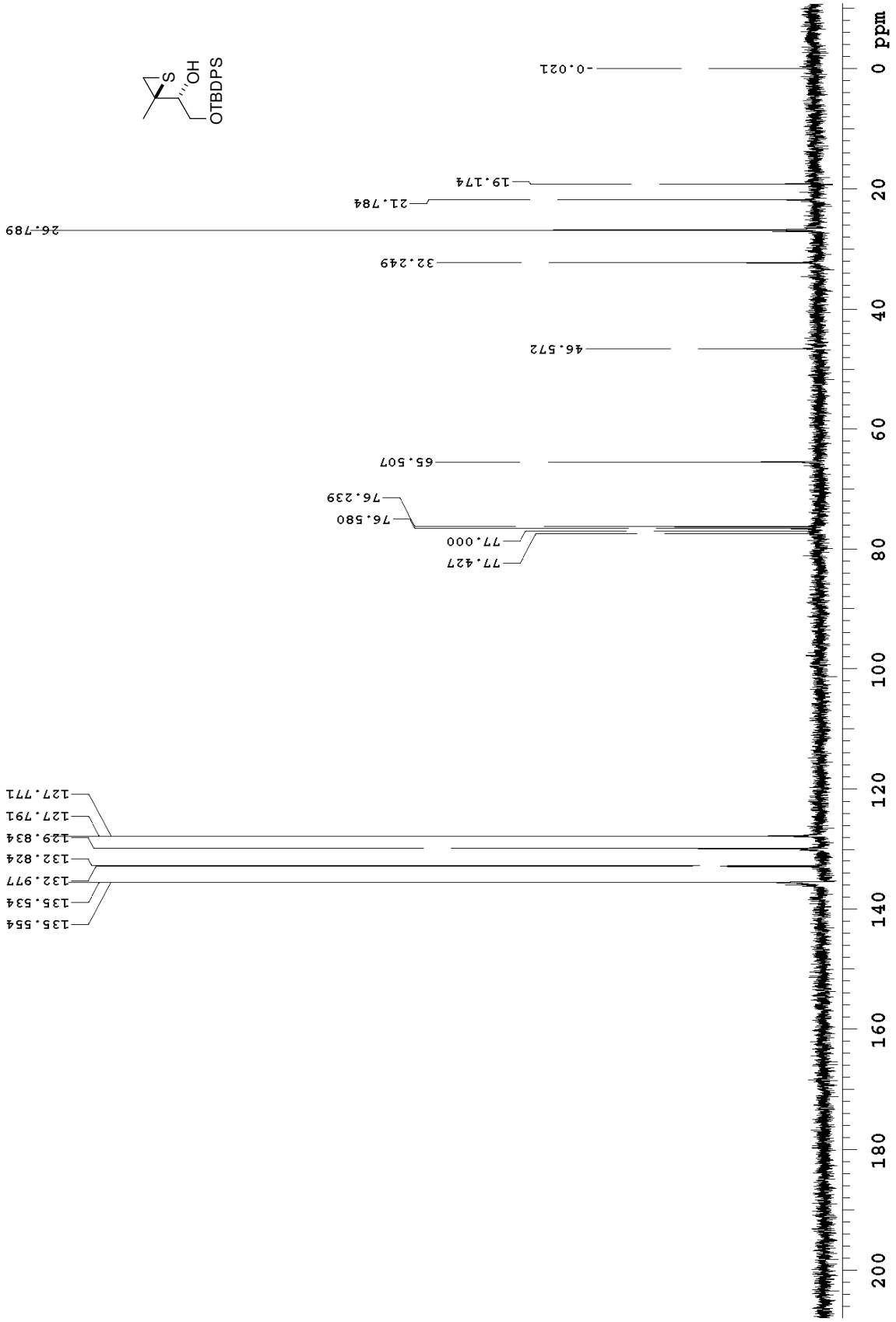

Br
O
OTBDPS
0.000
1.291
1.065
3.805
3.788
3.783
3.766
3.367
3.333
3.268
3.233
3.167
3.149
3.131
7.702
7.693
7.673
7.415
7.388
ppm
9.40
3.07
2.09
0.98
2.08
4.02
5.93

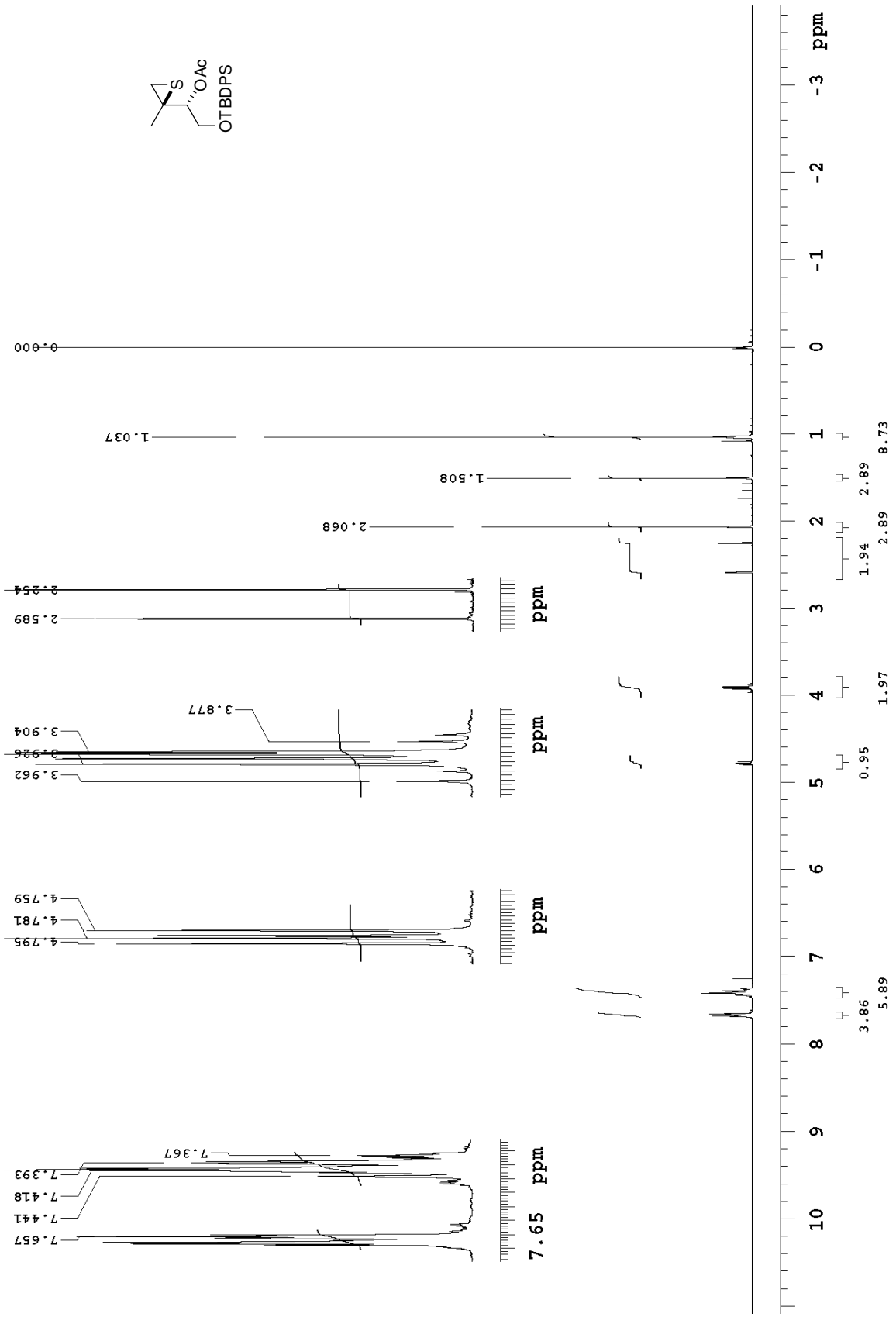

Br
O
OTBDPS
-0.015
15.149
19.154
26.689
39.123
59.293
62.430
64.225
76.580
77.000
77.420
135.540
135.514
133.178
132.957
129.820
127.758
0 ppm
20
40
60
80
100
120
140
160
180
200

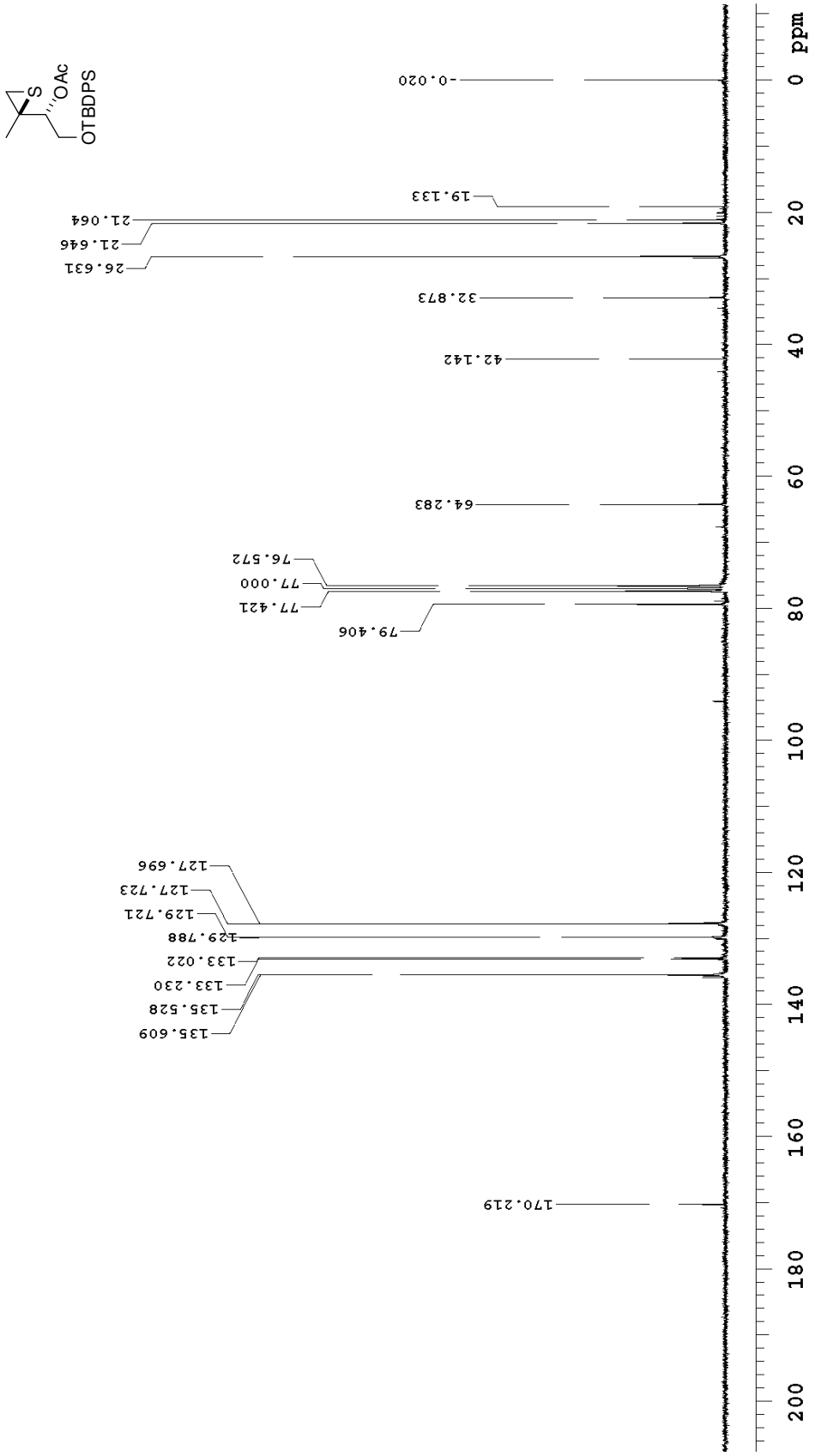

OTBDPS
0.000
1.057
1.153
2.356
2.999
3.045
3.105
3.129
3.151
3.688
3.706
3.726
3.744
3.764
3.782
3.802
3.820
7.387
7.389
7.407
7.409
7.414
7.662
7.436
7.414
7.389
ppm
9.13
2.91
2.92
2.92
2.01
3.96
5.87

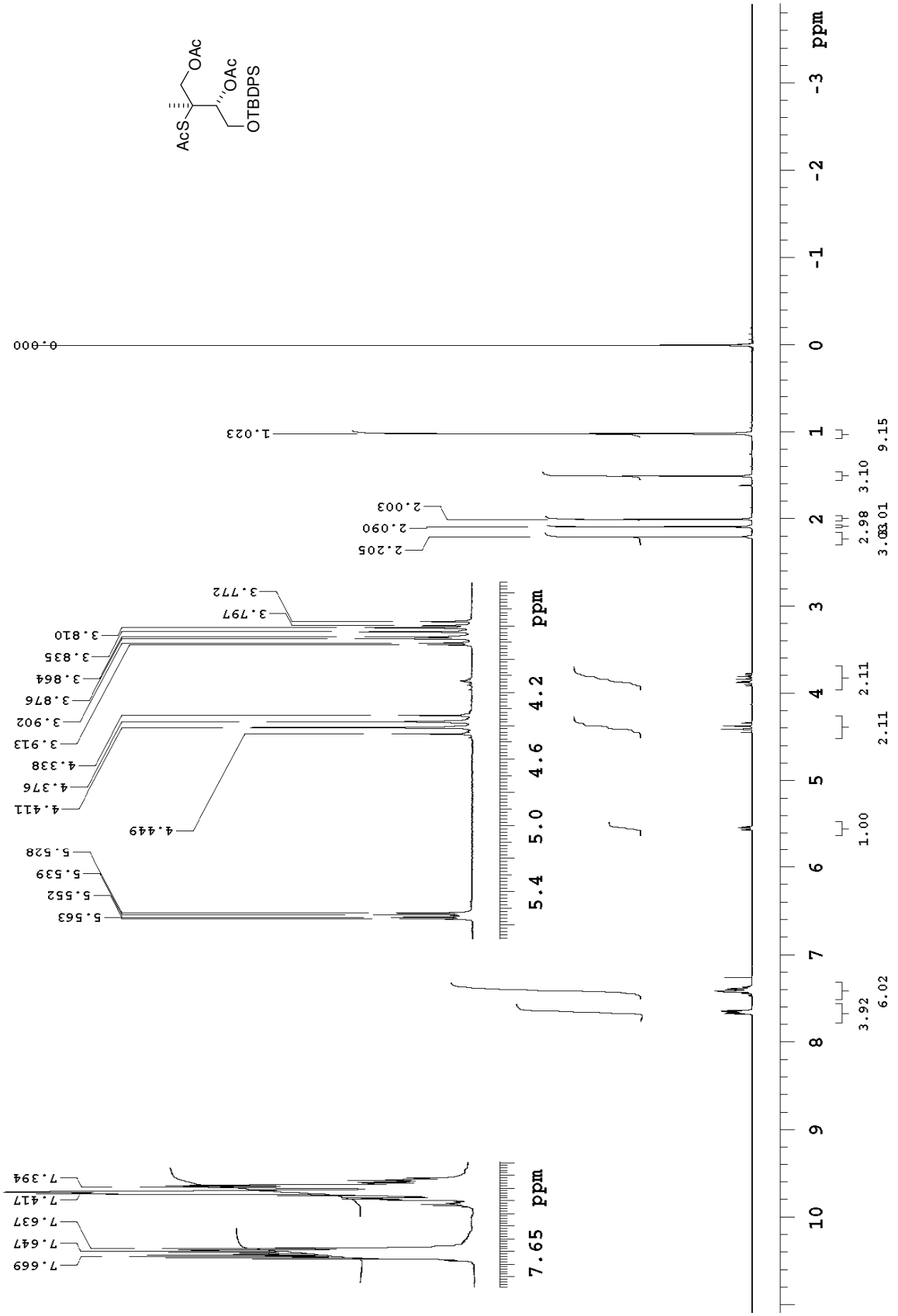

194.508
135.540
135.507
133.271
133.037
129.774
127.725
77.427
77.000
76.580
62.850
62.403
59.260
36.667
30.420
26.682
19.147
16.017
-0.028
OTBDPS
O
S
O
200
180
160
140
120
100
80
60
40
20
0 ppm

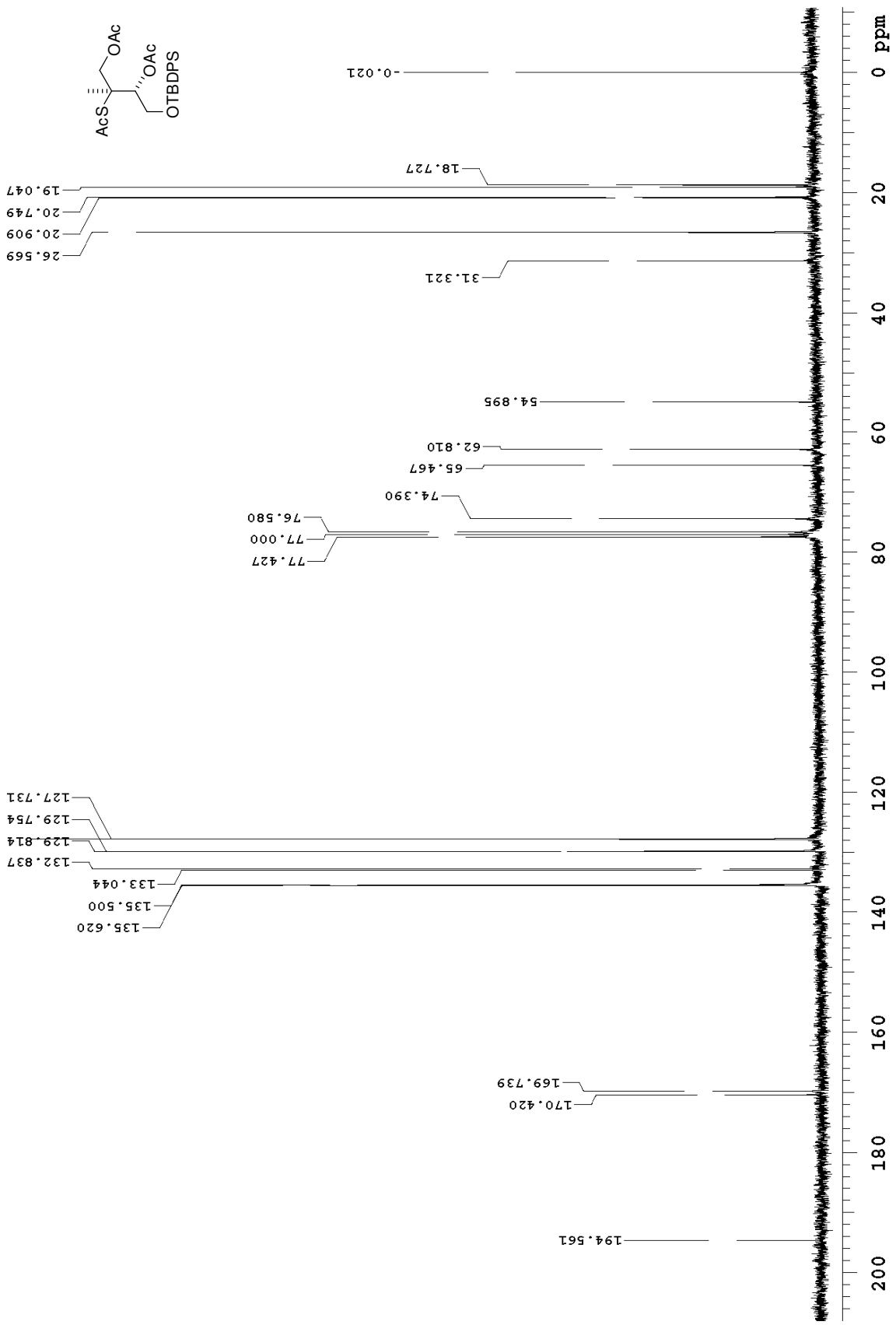

S
OH
OTBDPS
0.000
1.071
1.572
2.344
2.597
2.746
2.755
3.522
3.534
3.542
3.550
3.554
3.562
3.777
3.796
3.812
3.831
3.845
3.858
3.880
3.893
7.373
7.398
7.423
7.444
7.663
7.683
ppm

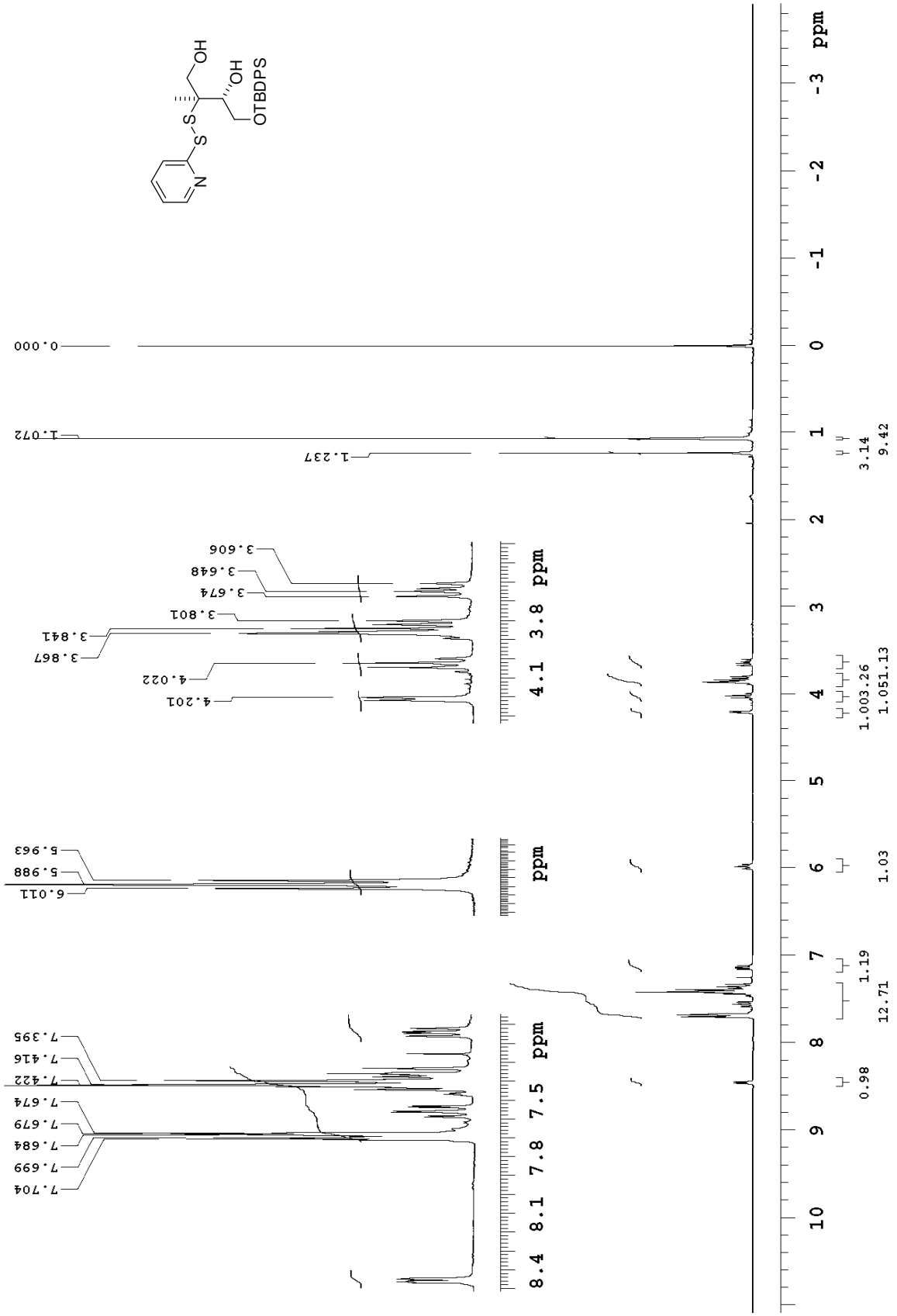

135.554
135.534
132.977
132.824
129.834
127.791
127.771
77.427
77.000
76.580
76.239
65.507
46.572
32.249
26.789
21.784
19.174
-0.021
OTBDPS
OH
S
0 ppm
20
40
60
80
100
120
140
160
180
200

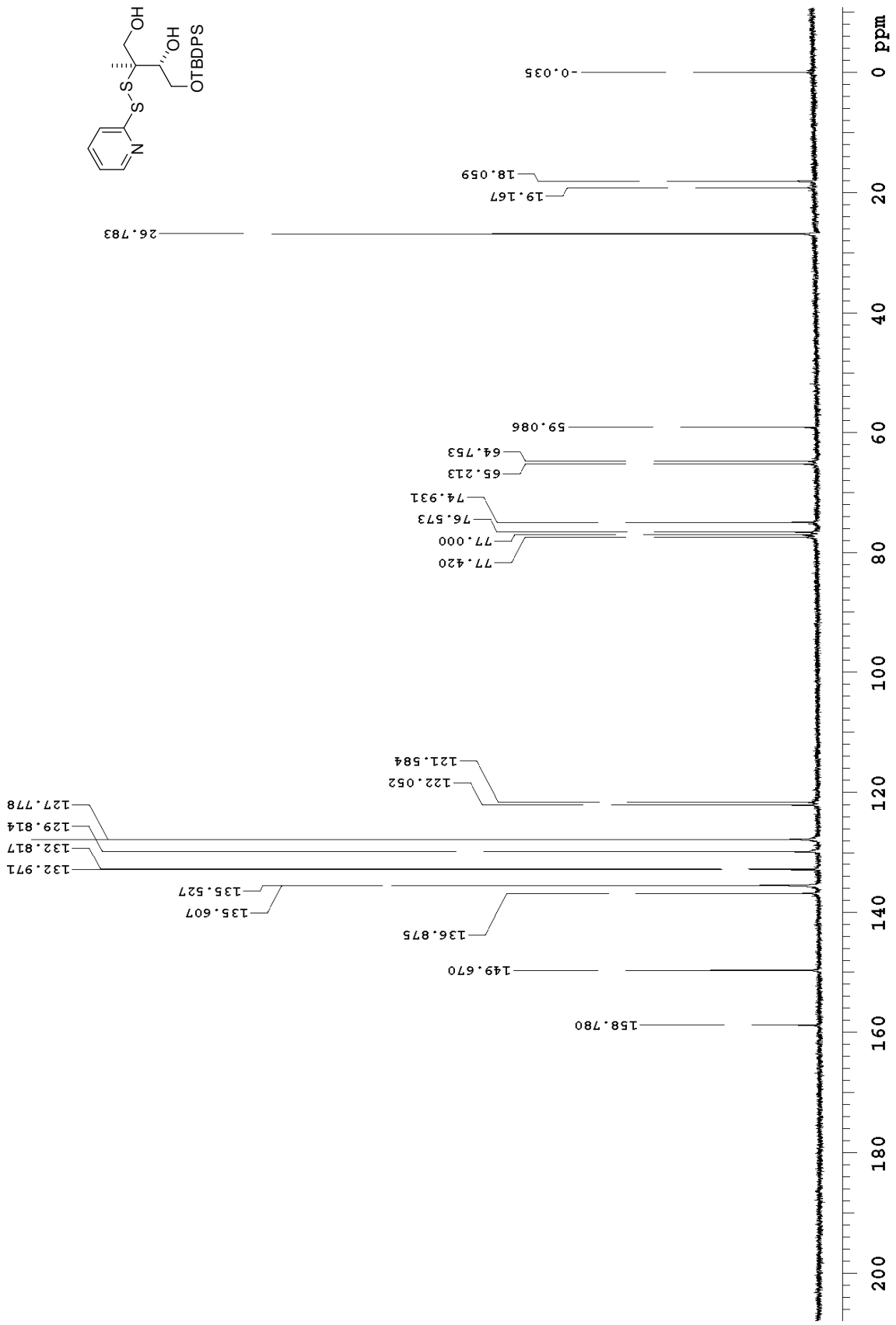

OTBDPS
OAc
S
7.657
7.441
7.418
7.393
7.367
7.65 ppm
4.795
4.781
4.759
3.962
3.926
3.904
3.877
2.589
2.254
2.068
1.508
1.037
0.000
3.86
5.89
0.95
1.97
1.94
2.89
2.89
8.73
ppm

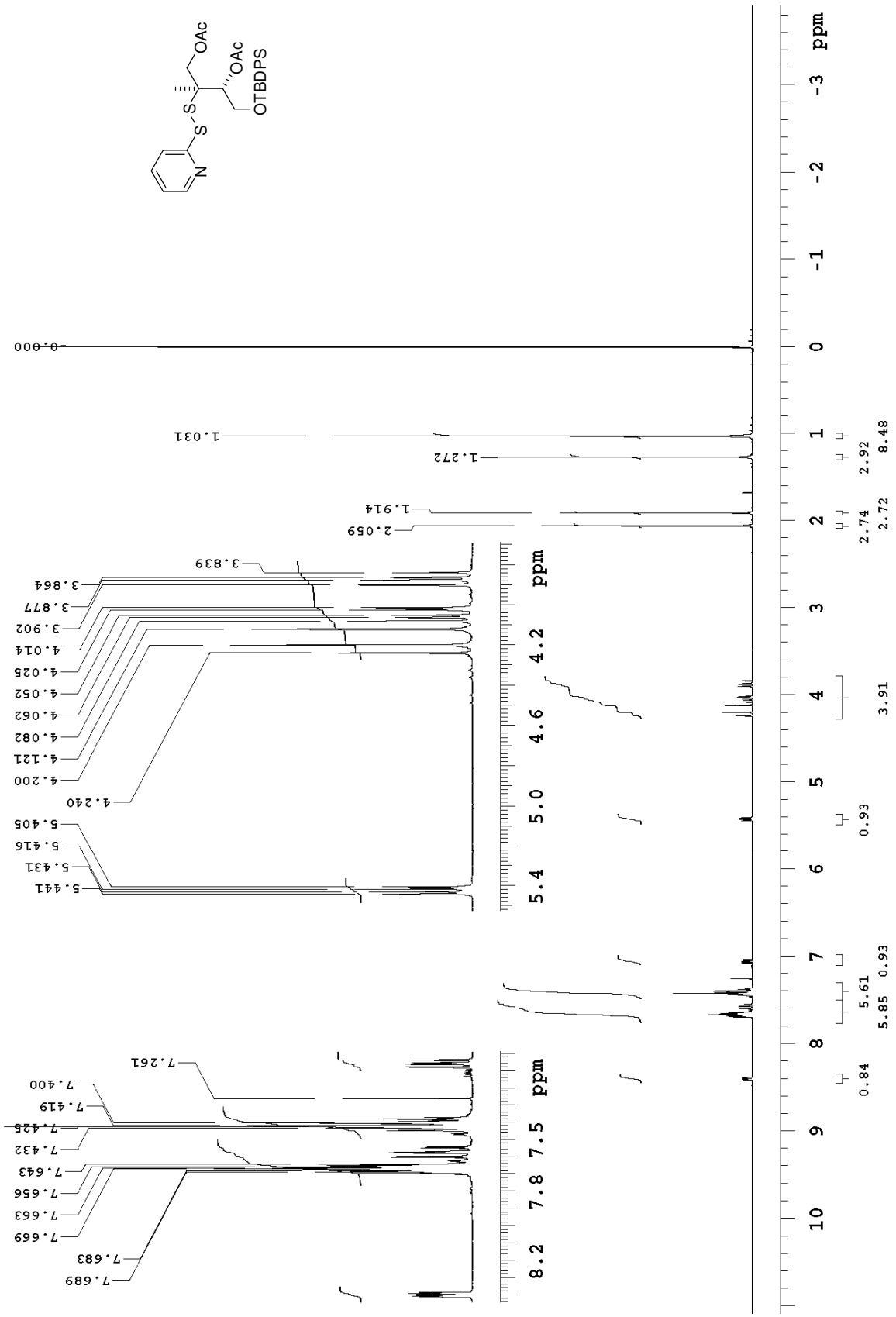

-0.020
19.133
21.064
21.646
26.631
32.873
42.142
64.283
76.572
77.000
77.421
79.406
127.696
127.723
129.721
129.788
133.022
133.230
135.528
135.609
170.219
0 ppm
20
40
60
80
100
120
140
160
180
200
S
OAc
OTBDPS

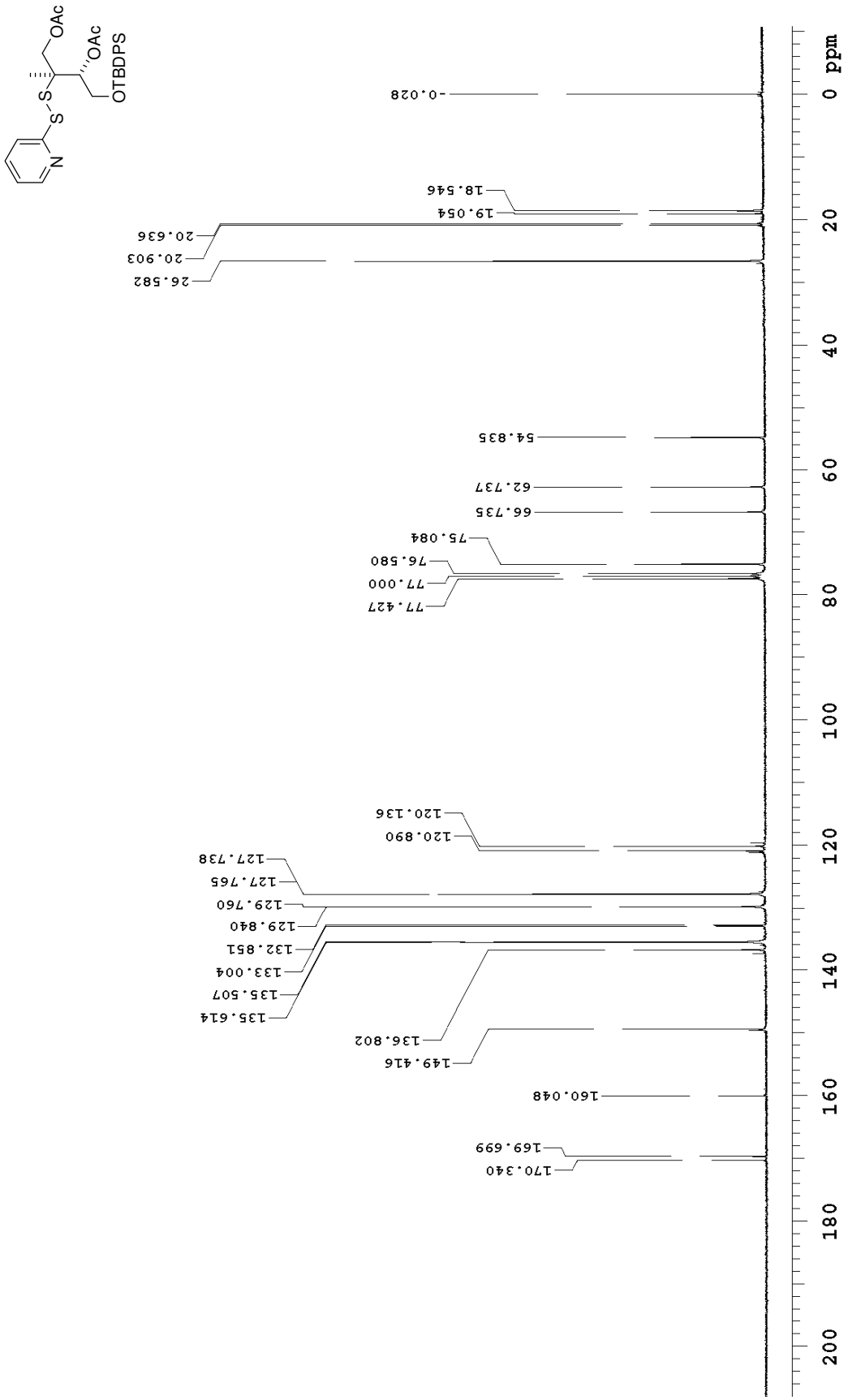

AcS
OAc
OAc
OTBDPS
0.000
1.023
2.003
2.090
2.205
3.772
3.797
3.810
3.835
3.864
3.876
3.902
3.913
4.338
4.376
4.411
4.449
5.528
5.539
5.552
5.563
7.394
7.417
7.637
7.647
7.669
ppm

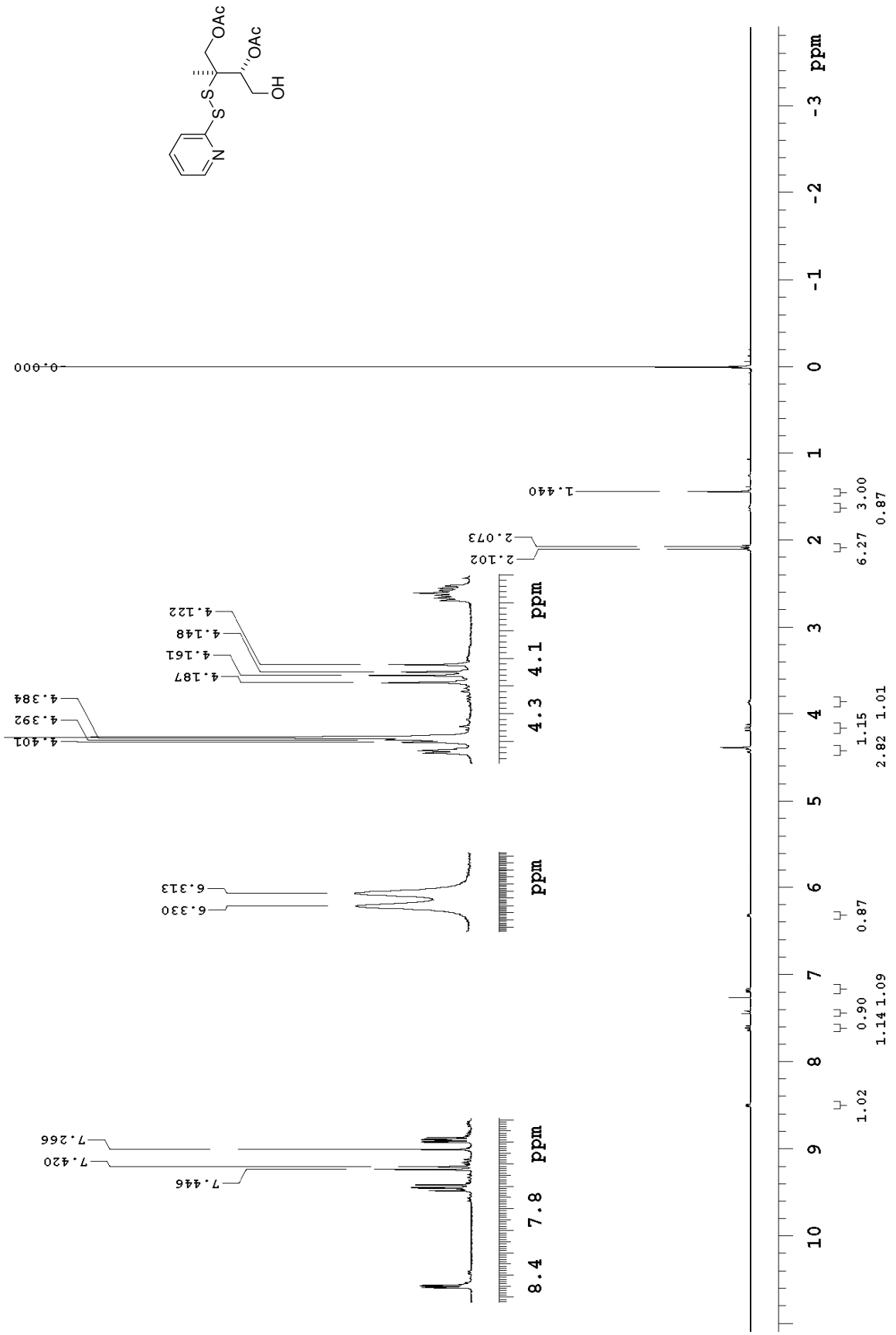

AcS
OAc
OAc
OTBDPS
-0.021
18.727
19.047
20.749
20.909
26.569
31.321
54.895
62.810
65.467
74.390
76.580
77.000
77.427
127.731
129.754
129.814
132.837
133.044
135.500
135.620
169.739
170.420
194.561
0 ppm
20
40
60
80
100
120
140
160
180
200

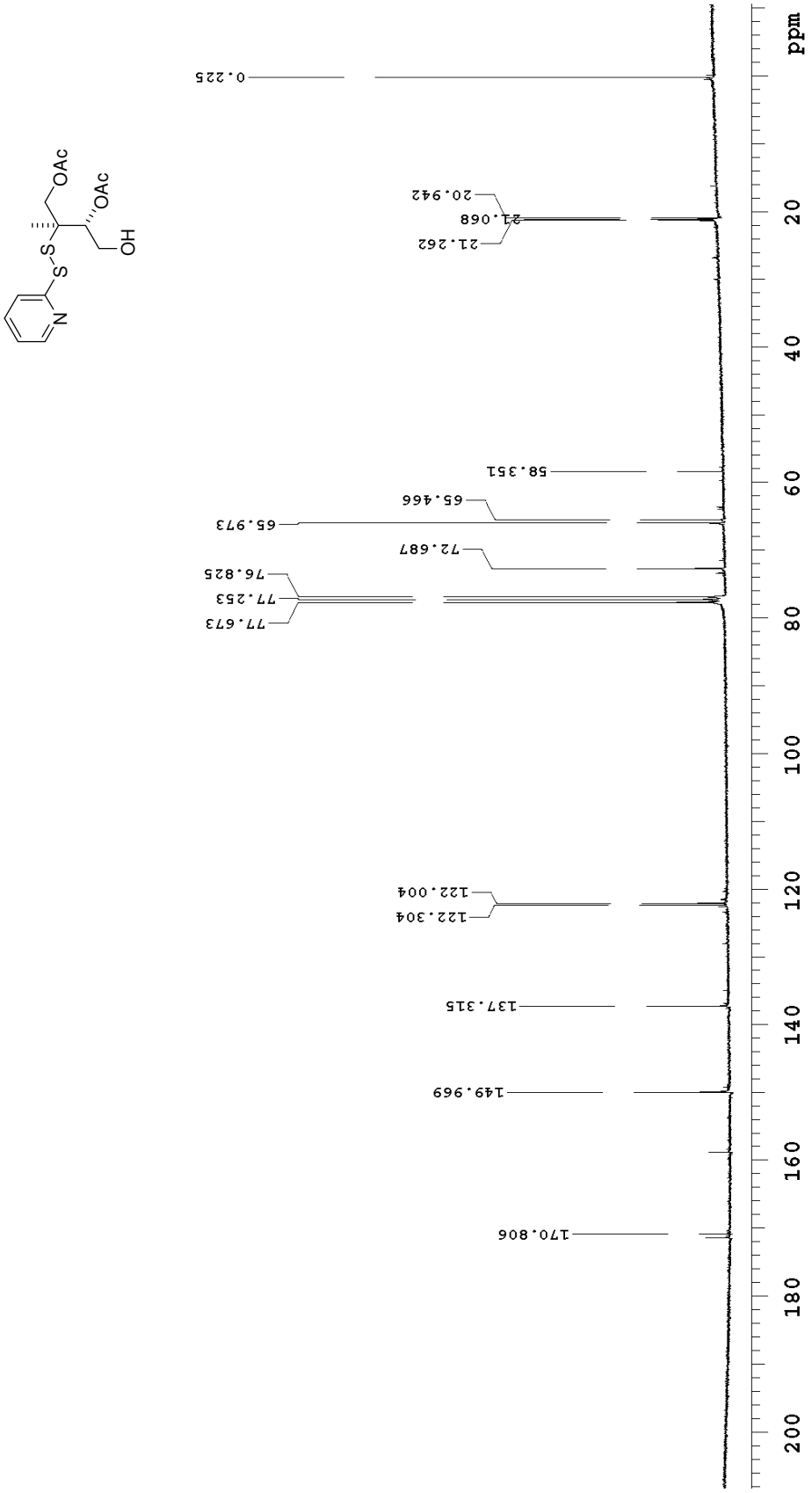

OH
OH
OTBDPS
S
S
N
0.000
1.072
1.237
3.606
3.648
3.674
3.801
3.841
3.867
4.022
4.201
6.011
5.988
5.963
7.704
7.699
7.684
7.679
7.674
7.422
7.416
7.395
ppm
ppm
ppm
0.98
1.19
12.71
1.03
1.003.26
1.051.13
3.14
9.42

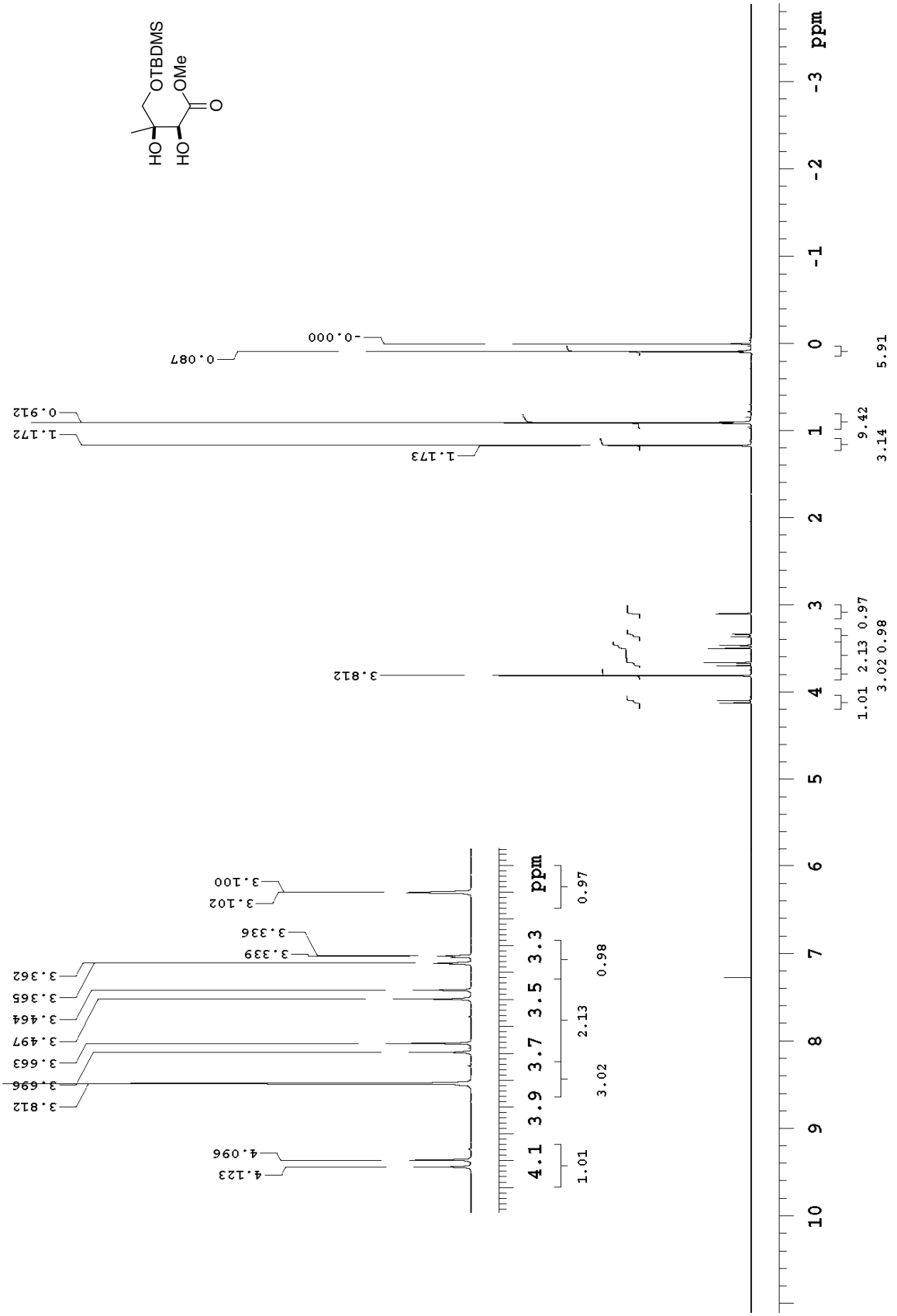

OH
OH
OTBDPS
S
S
N
-0.035
18.059
19.167
26.783
59.086
64.753
65.213
74.931
76.573
77.000
77.420
121.584
122.052
127.778
129.814
132.817
132.971
135.527
135.607
136.875
149.670
158.780
0 ppm
20
40
60
80
100
120
140
160
180
200

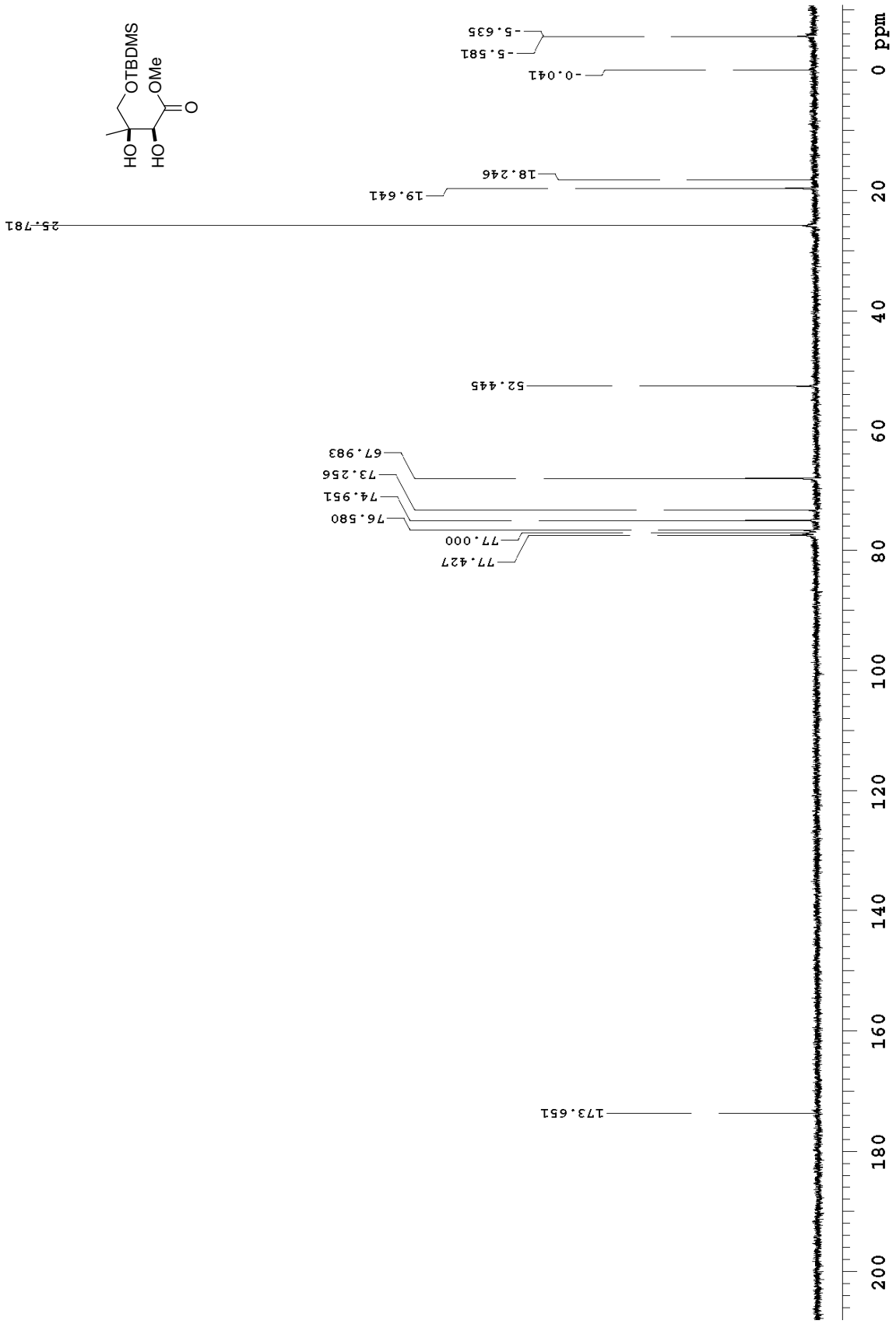

OAc
OAc
OTBDPS
S
S
N
0.000
1.031
1.272
1.914
2.059
3.839
3.864
3.877
3.902
4.014
4.025
4.052
4.062
4.082
4.121
4.200
4.240
5.405
5.416
5.431
5.441
7.261
7.400
7.419
7.425
7.432
7.643
7.656
7.663
7.669
7.683
7.689
ppm
ppm
ppm
ppm
0.84
5.61
5.85
0.93
0.93
3.91
2.74
2.72
2.92
8.48

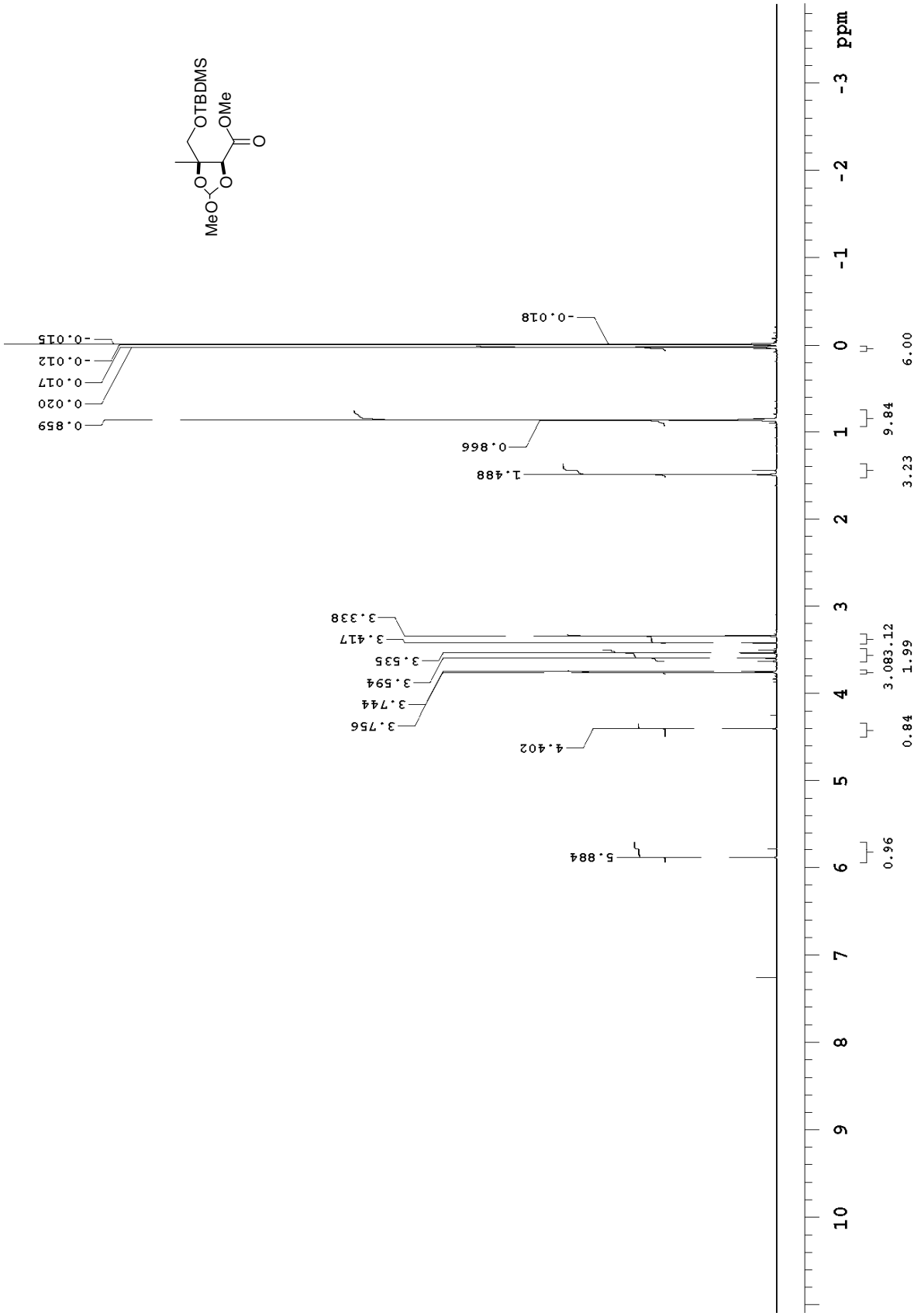

OAc
OAc
OTBDPS
S
S
N
-0.028
18.546
19.054
20.636
20.903
26.582
54.835
62.737
66.735
75.084
76.580
77.000
77.427
120.136
120.890
127.738
127.765
129.760
129.840
132.851
133.004
135.507
135.614
136.802
149.416
160.048
169.699
170.340
0 ppm
20
40
60
80
100
120
140
160
180
200

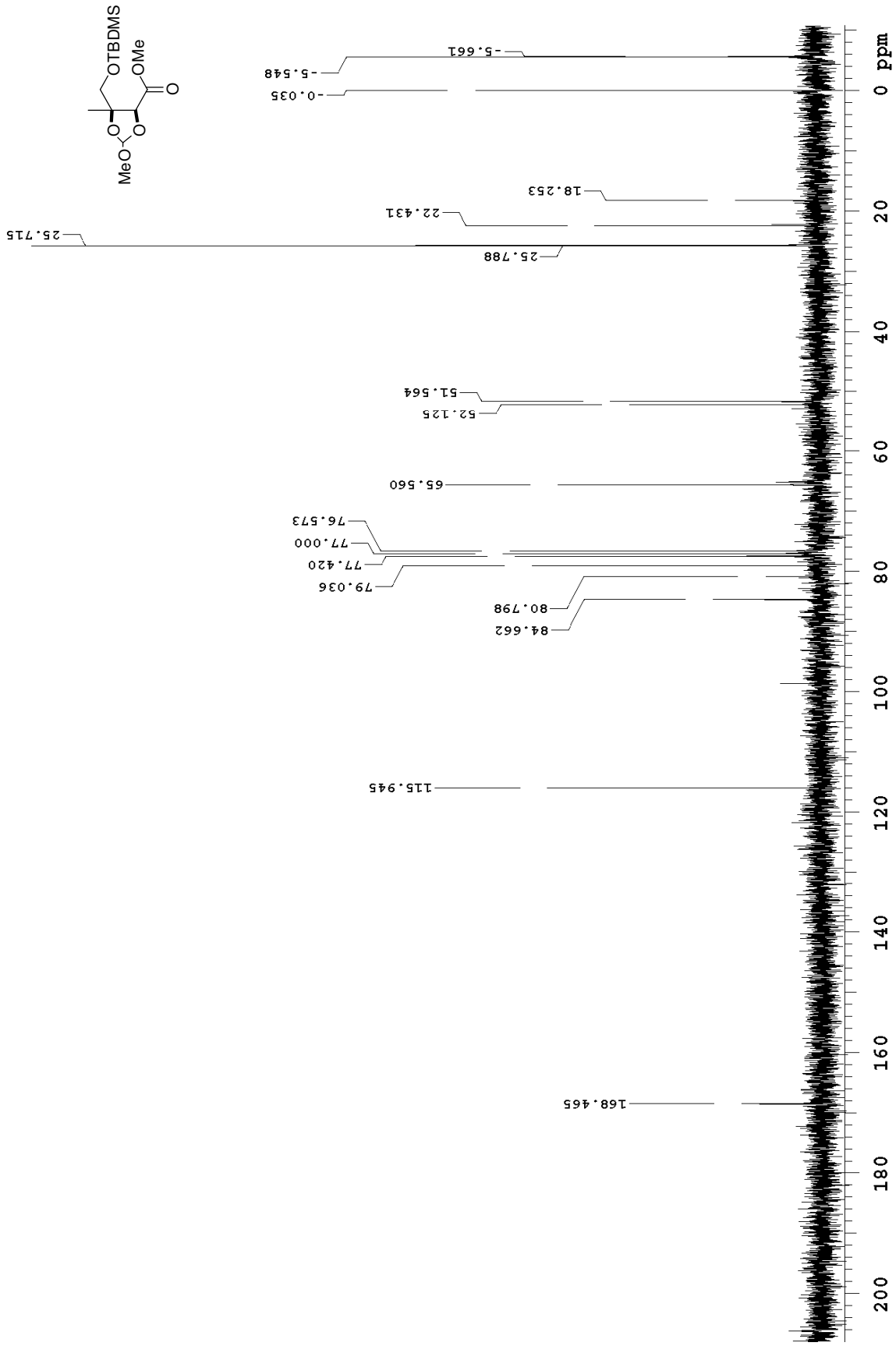

OAc
OAc
OH
S
S
N
0.000
1.440
2.073
2.102
4.122
4.148
4.161
4.187
4.384
4.392
4.401
6.313
6.330
7.266
7.420
7.446
ppm
ppm
ppm
1.02
0.90
1.14
1.09
0.87
1.15
2.82
1.01
6.27
3.00
0.87

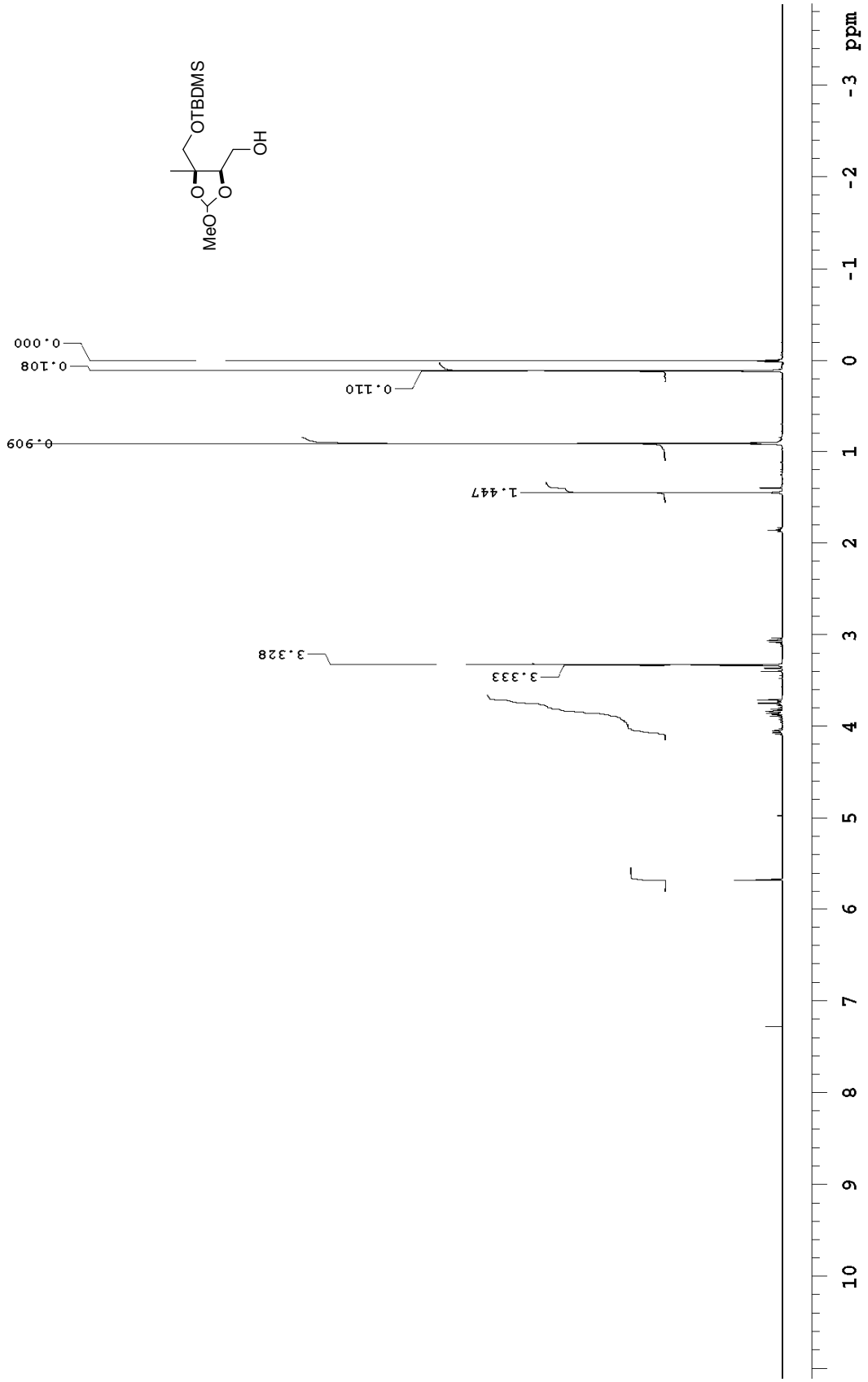

OAc
OAc
OH
S
S
N
0.225
20.942
21.068
21.262
58.351
65.466
65.973
72.687
76.825
77.253
77.673
122.004
122.304
137.315
149.969
170.806
ppm

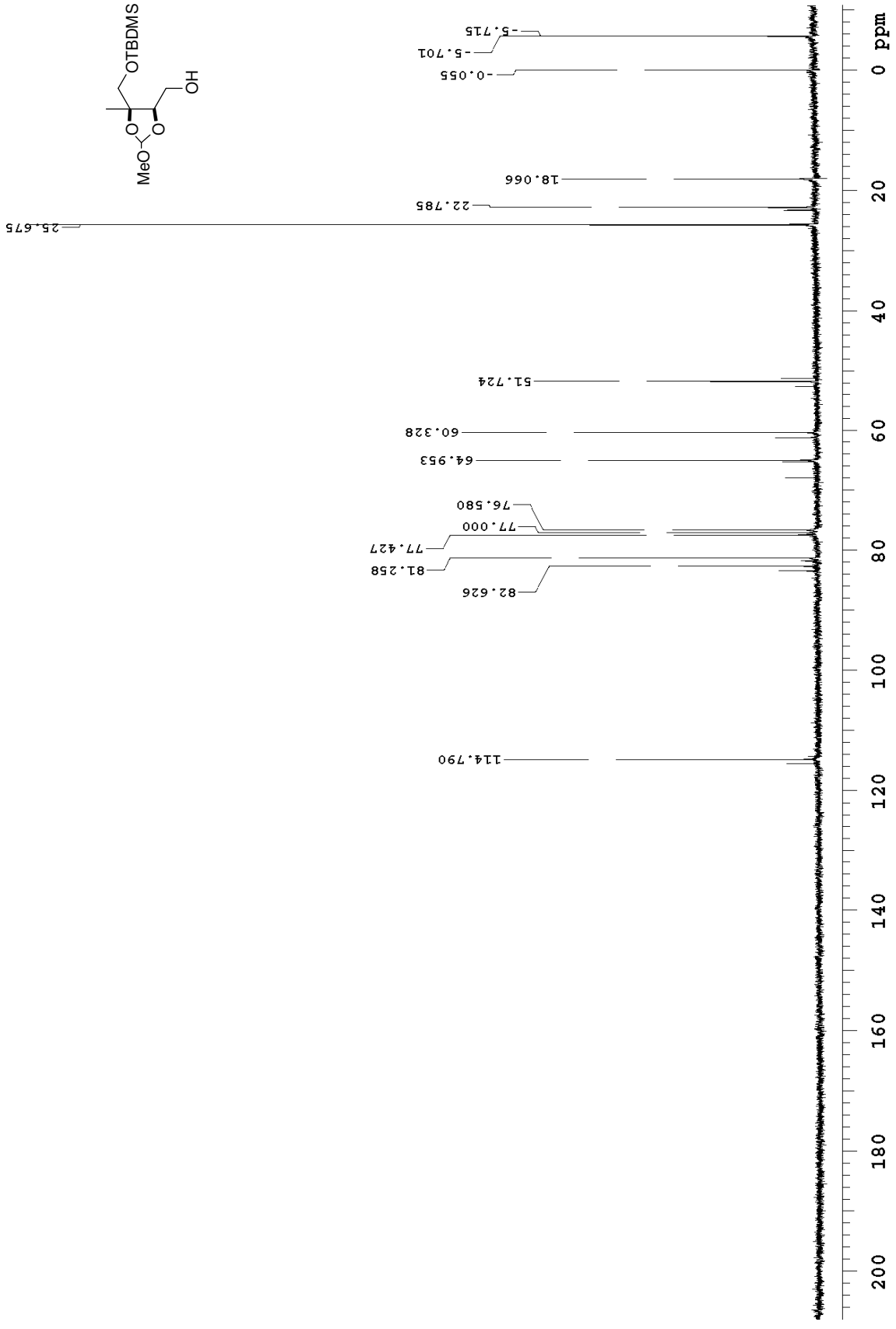

OTBDMS
OMe
O
HO
HO
-0.000
0.087
0.912
1.172
1.173
3.812
4.123
4.096
3.812
3.696
3.663
3.497
3.464
3.365
3.362
3.339
3.336
3.102
3.100
ppm
1.01
2.13
0.97
3.02
0.98
9.42
3.14
5.91

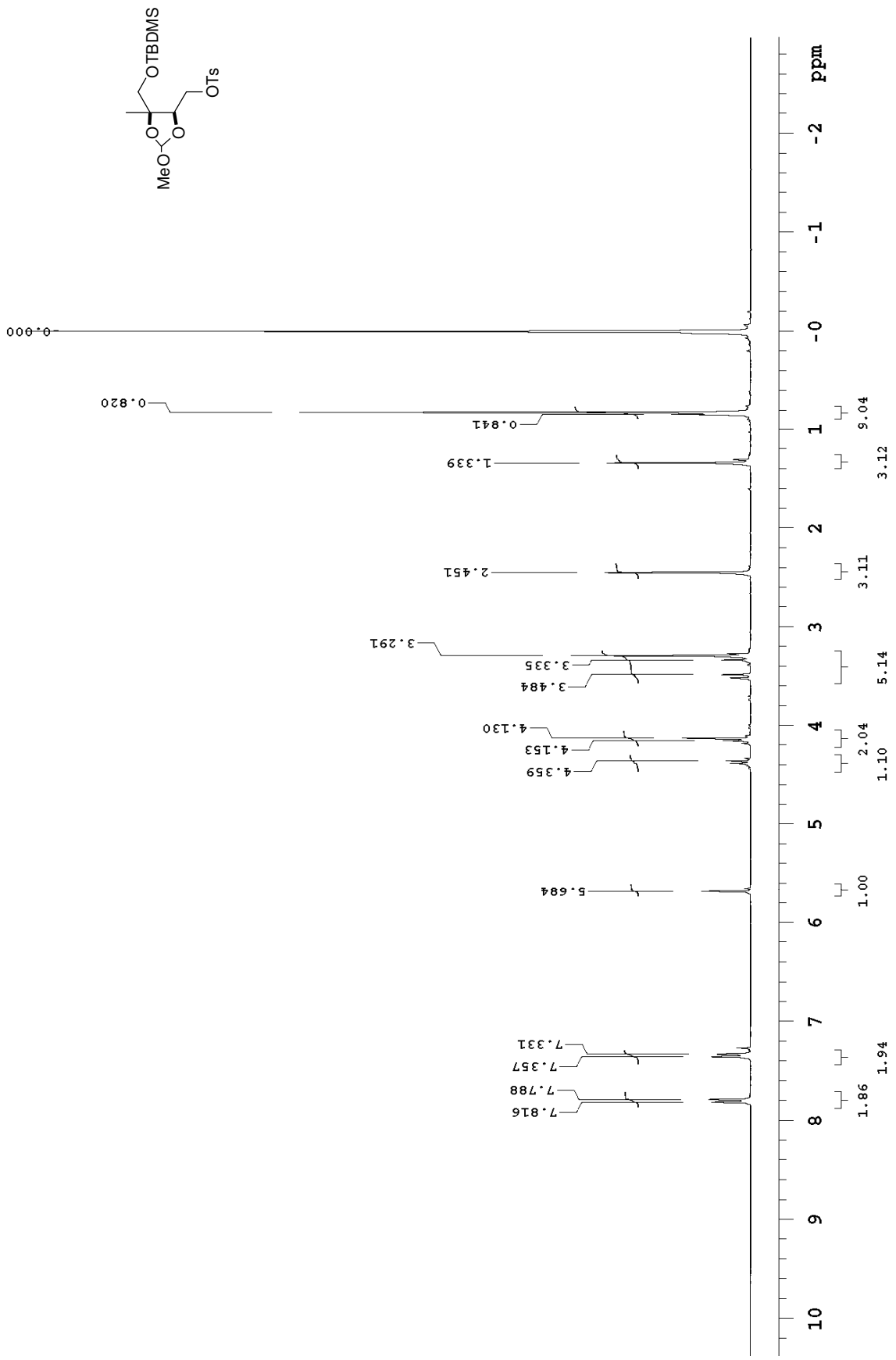

OTBDMS
OMe
O
HO
HO
-5.635
-5.581
-0.041
18.246
19.641
25.781
52.445
67.983
73.256
74.951
76.580
77.000
77.427
173.651
0 ppm
20
40
60
80
100
120
140
160
180
200

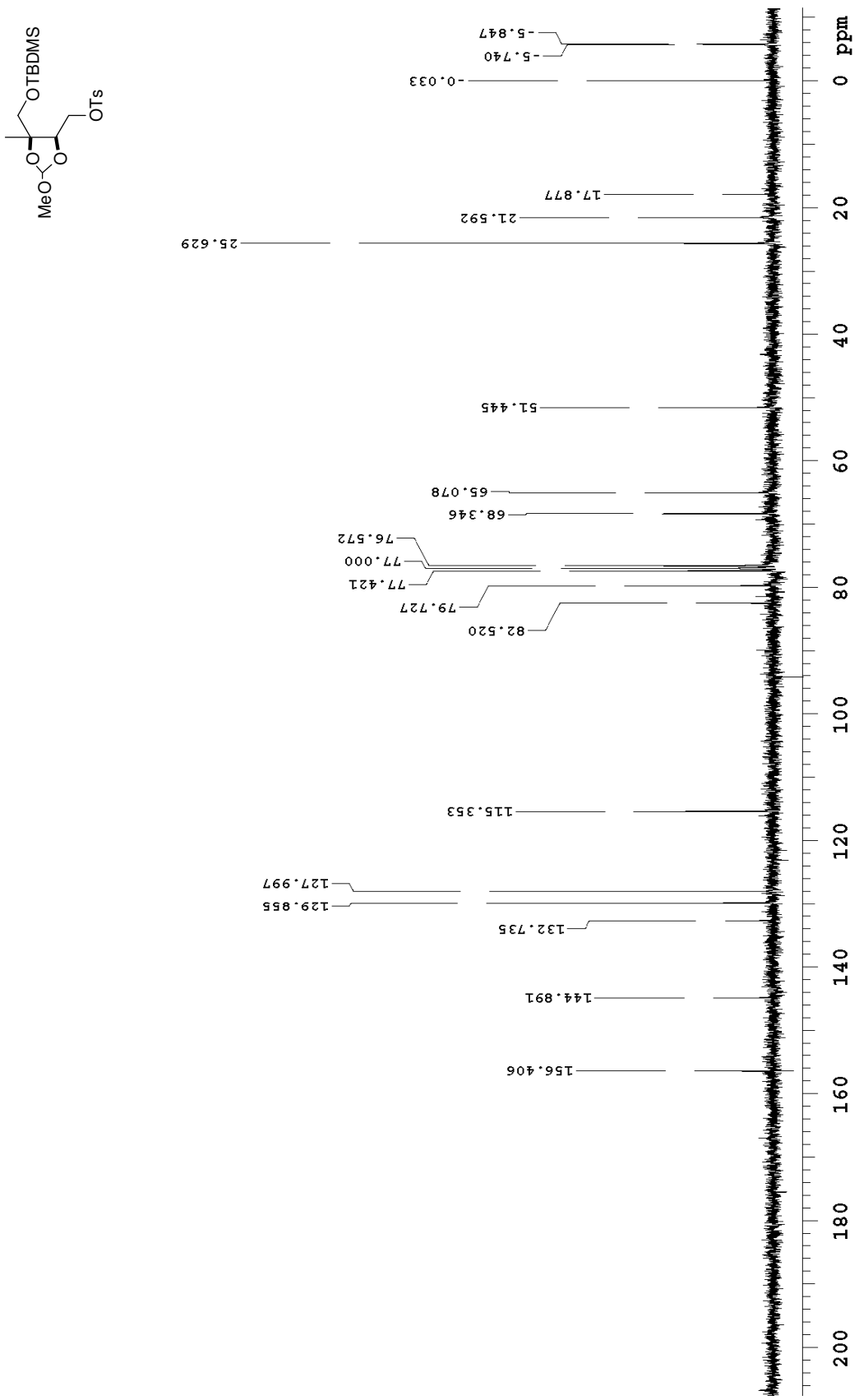

OTBDMS
OMe
O
O
O
MeO
-0.018
-0.015
-0.012
0.017
0.020
0.859
0.866
1.488
3.338
3.417
3.535
3.594
3.744
3.756
4.402
5.884
ppm
6.00
9.84
3.23
3.08
3.12
1.99
0.84
0.96

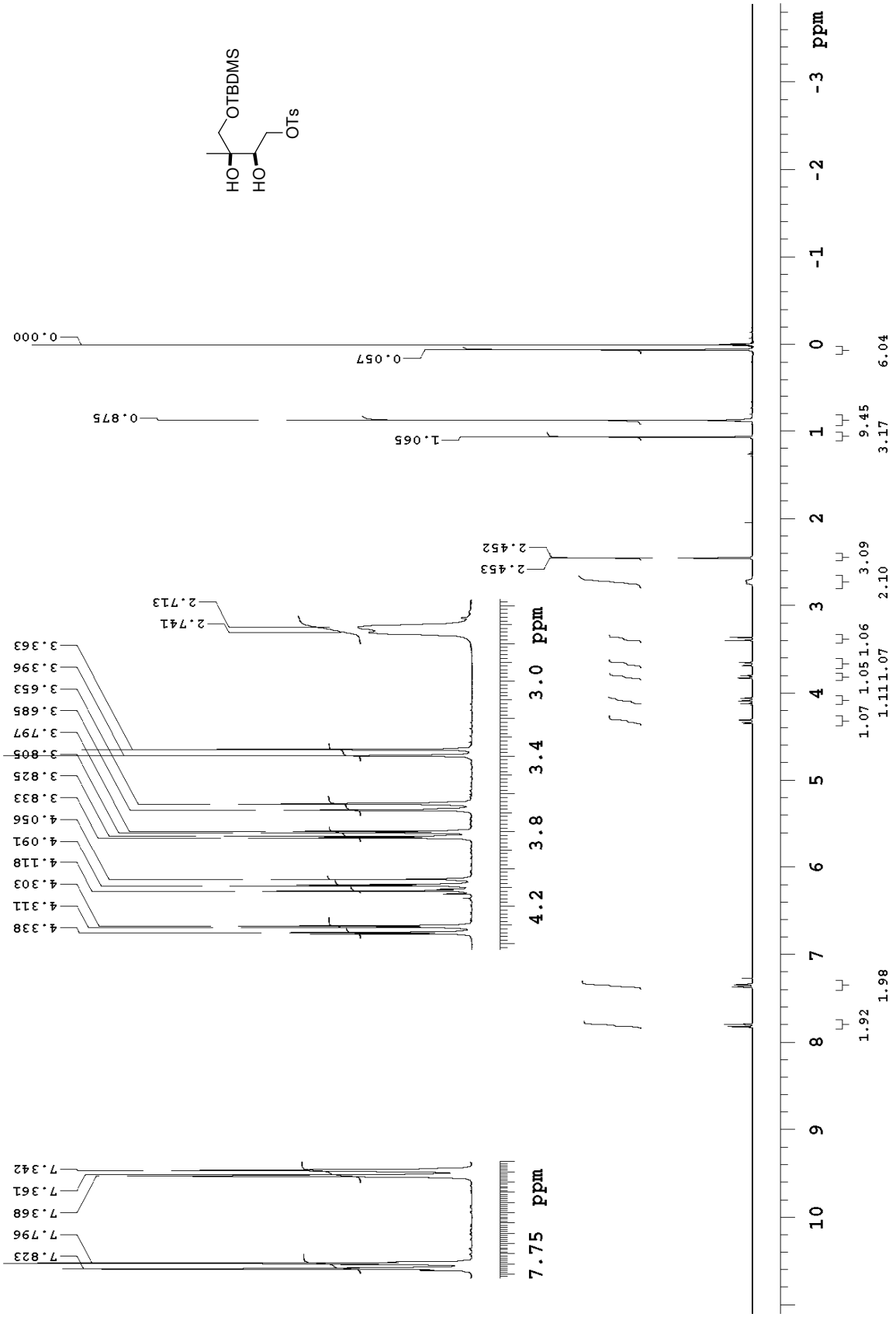

OTBDMS
OMe
O
O
O
MeO
-5.661
-5.548
-0.035
18.253
22.431
25.715
25.788
51.564
52.125
65.560
76.573
77.000
77.420
79.036
80.798
84.662
115.945
168.465
0 ppm
20
40
60
80
100
120
140
160
180
200

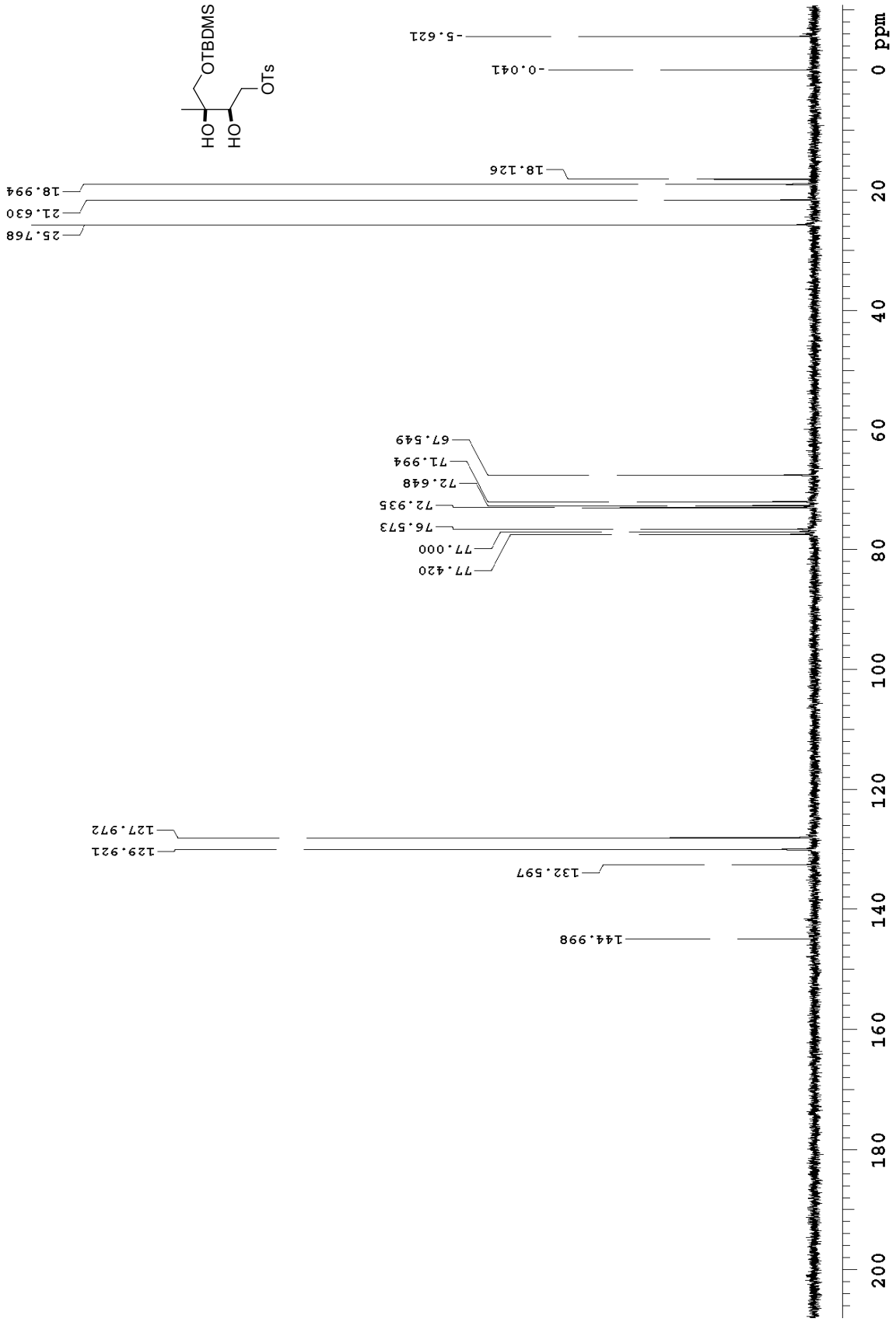

OTBDMS
OH
O
O
MeO
0.000
0.108
0.110
0.909
1.447
3.328
3.333
ppm

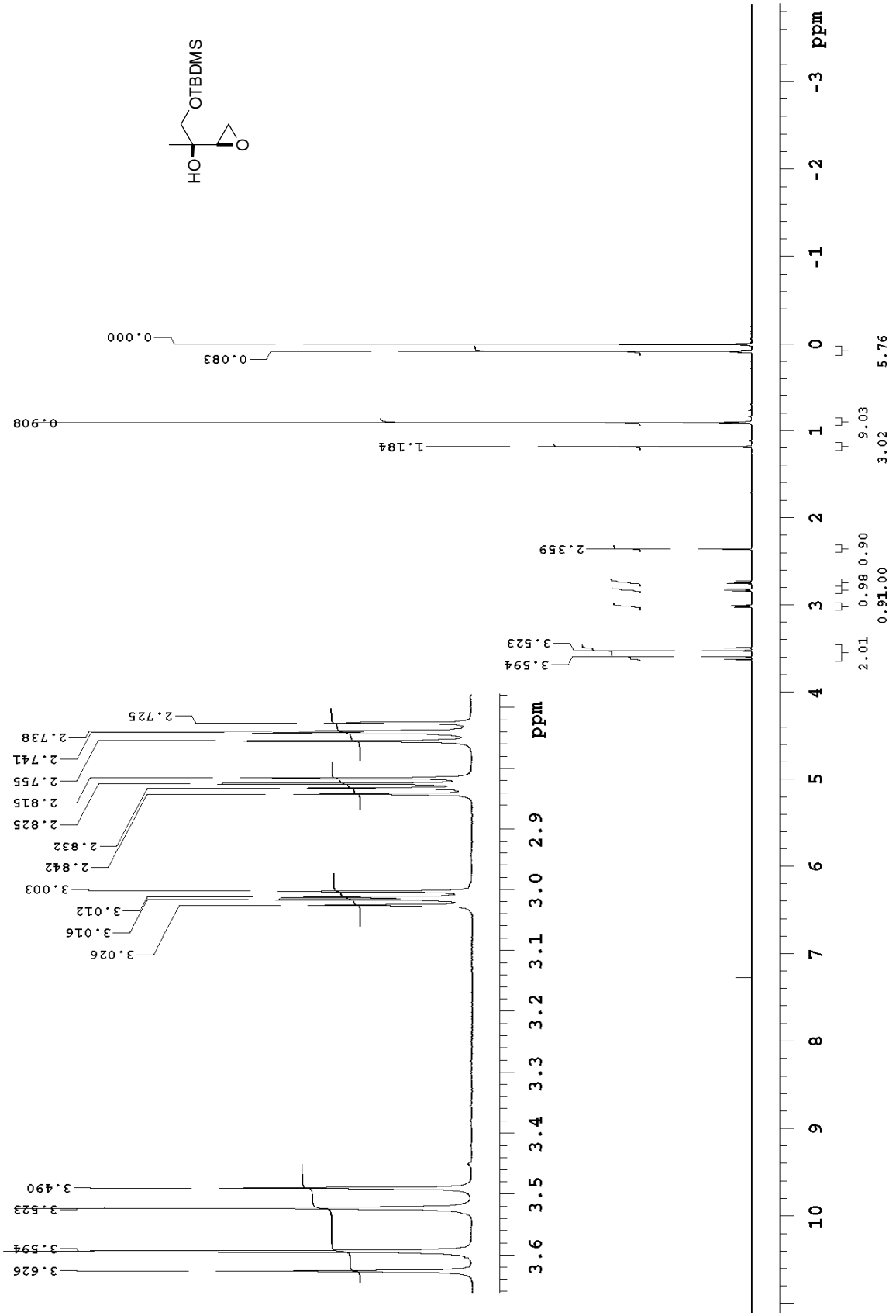

OTBDMS
OH
O
O
MeO
-5.715
-5.701
-0.055
18.066
22.785
25.675
51.724
60.328
64.953
76.580
77.000
77.427
81.258
82.626
114.790
0 ppm
20
40
60
80
100
120
140
160
180
200

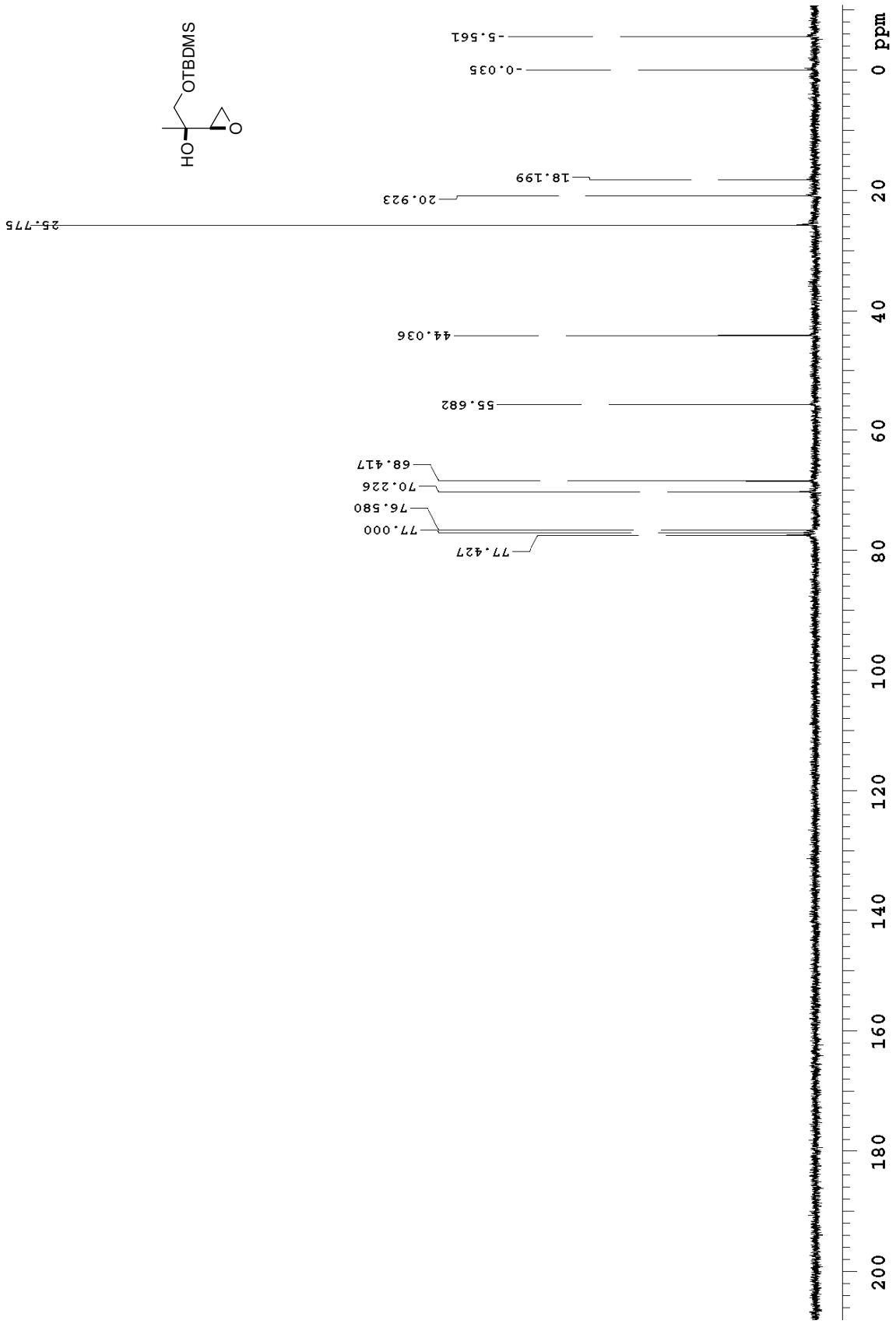

OTBDMS
OTs
MeO
O
O
O
0.000
0.820
0.841
1.339
2.451
3.291
3.335
3.484
4.130
4.153
4.359
5.684
7.331
7.357
7.788
7.816
9.04
3.12
3.11
5.14
2.04
1.10
1.00
1.94
1.86
ppm

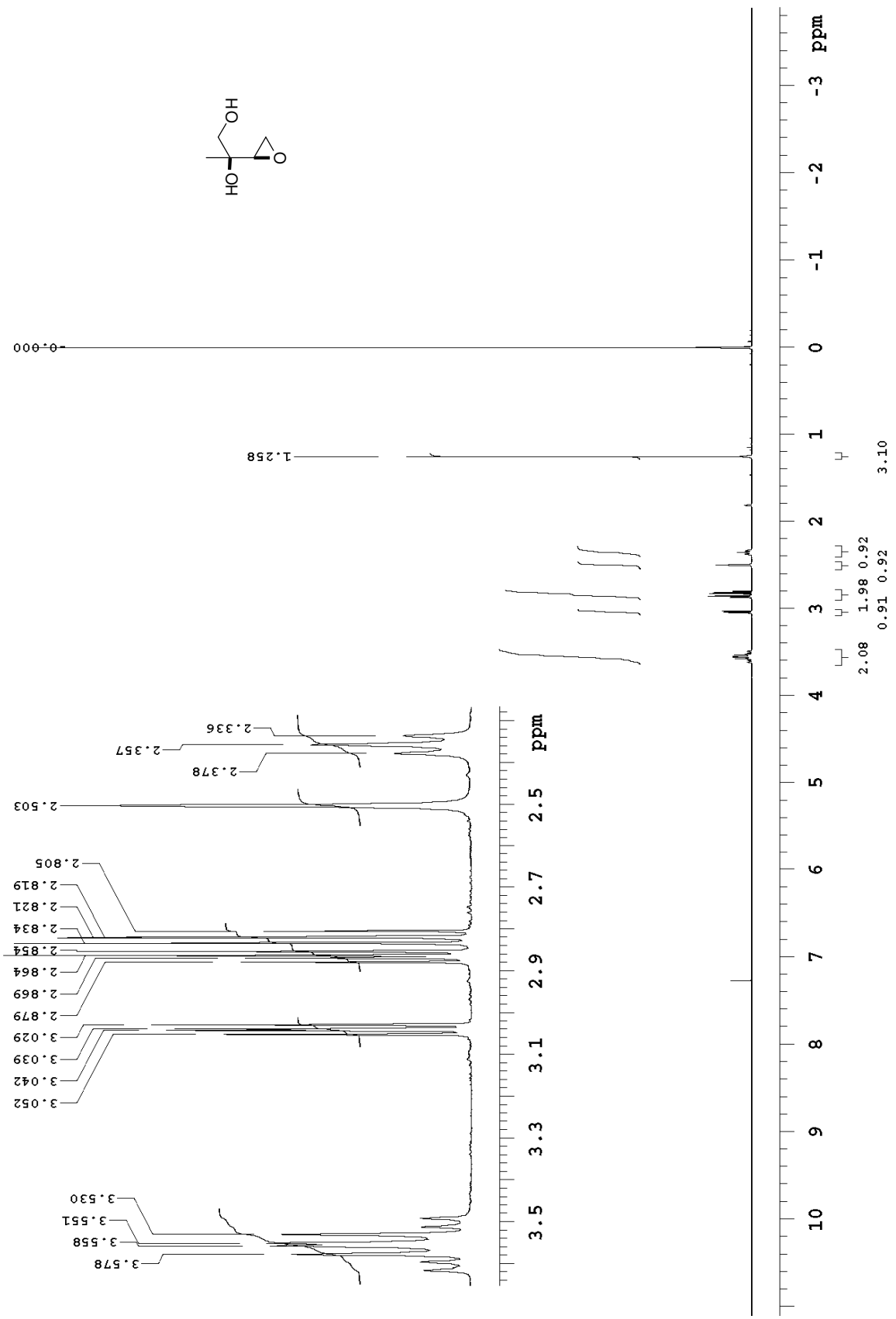

OTBDMS
OTs
MeO
O
O
O
-5.847
-5.740
-0.033
17.877
21.592
25.629
51.445
65.078
68.346
76.572
77.000
77.421
79.727
82.520
115.353
127.997
129.855
132.735
144.891
156.406
0
20
40
60
80
100
120
140
160
180
200
ppm

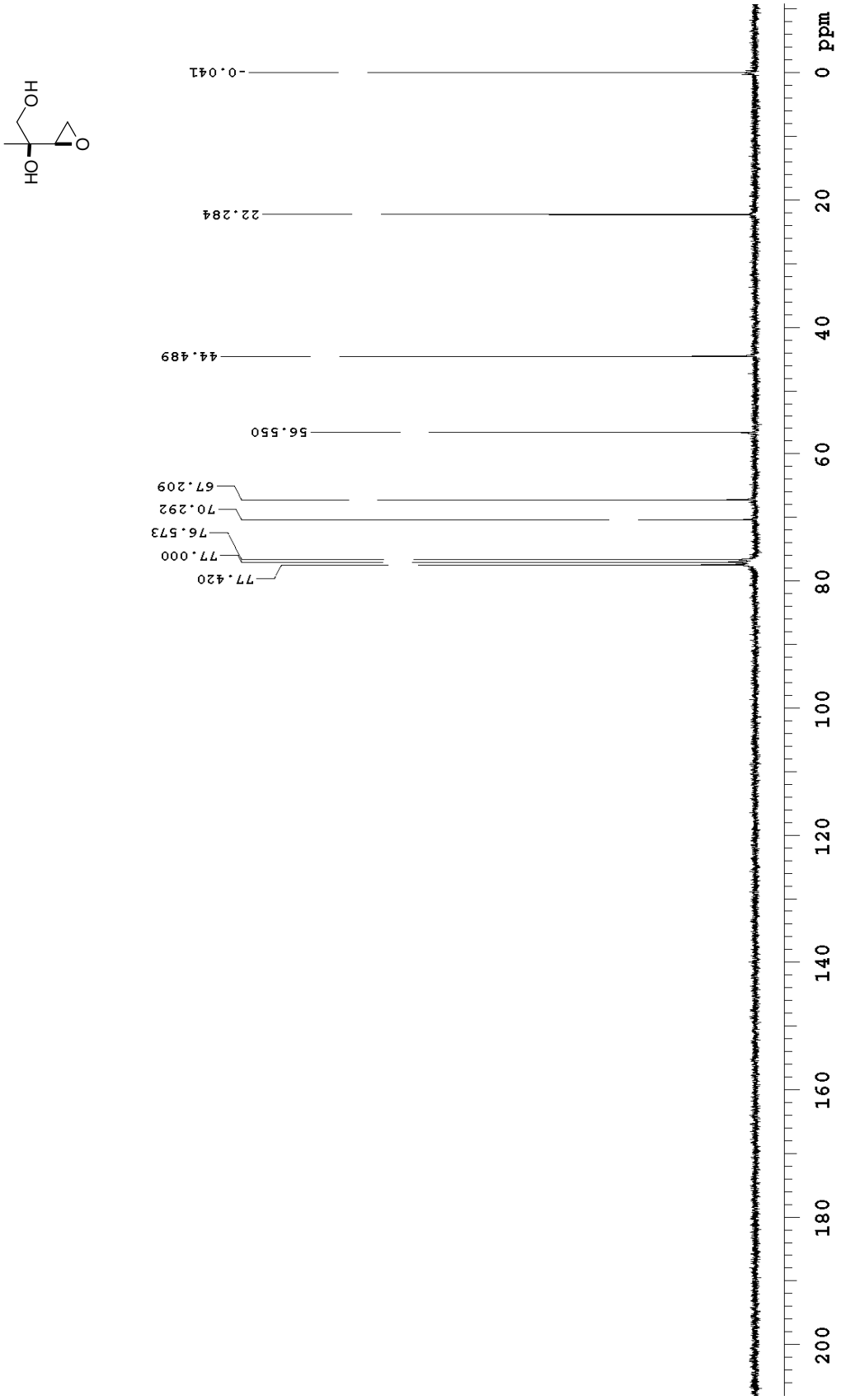

OTBDMS
OTs
HO
HO
0.000
0.057
0.875
1.065
2.452
2.453
2.713
2.741
3.363
3.396
3.653
3.685
3.797
3.805
3.825
3.833
4.056
4.091
4.118
4.303
4.311
4.338
7.342
7.361
7.368
7.796
7.823
ppm

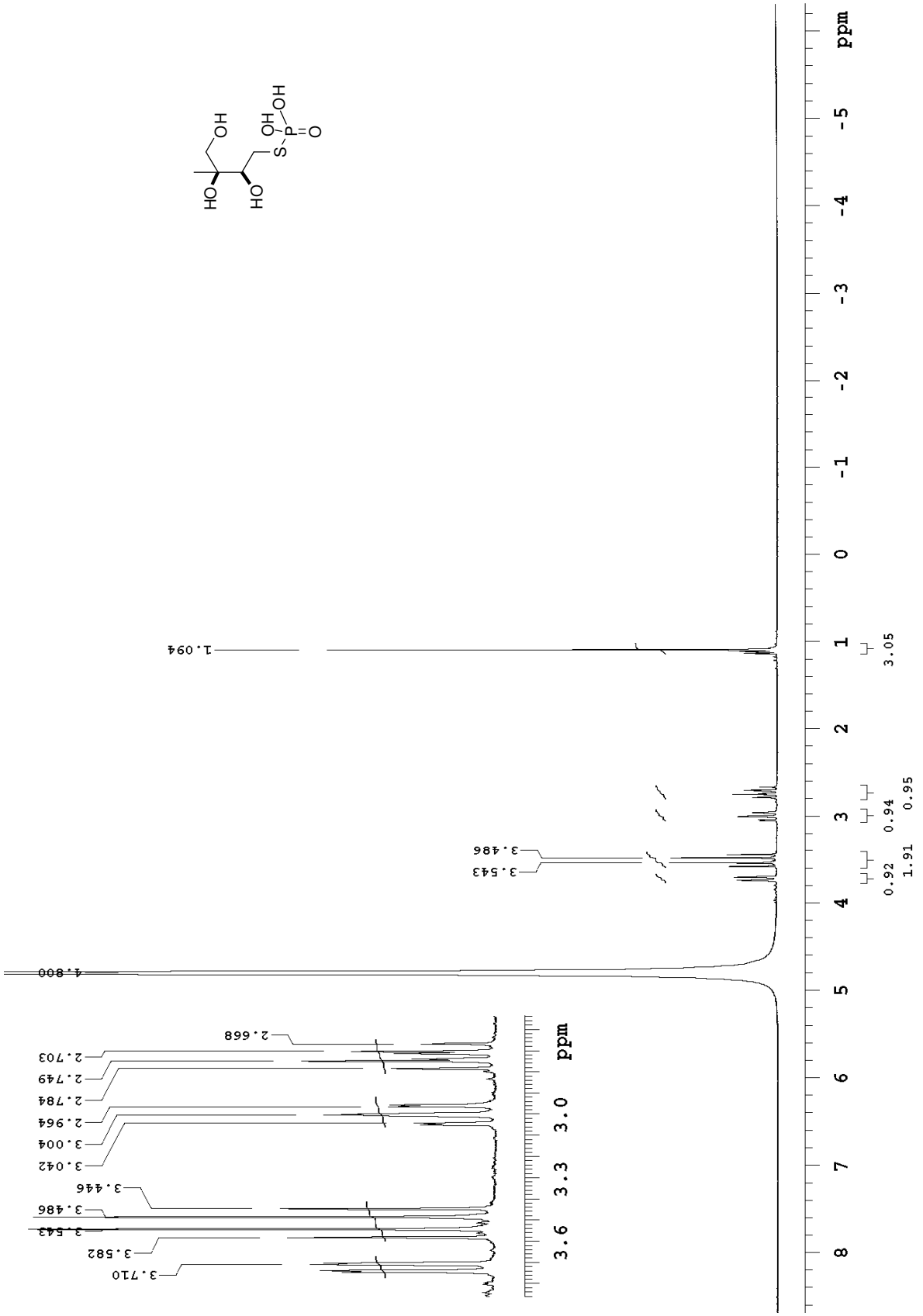

OTBDMS
OTs
HO
HO
-5.621
-0.041
18.126
18.994
21.630
25.768
67.549
71.994
72.648
72.935
76.573
77.000
77.420
127.972
129.921
132.597
144.998
0 ppm
20
40
60
80
100
120
140
160
180
200

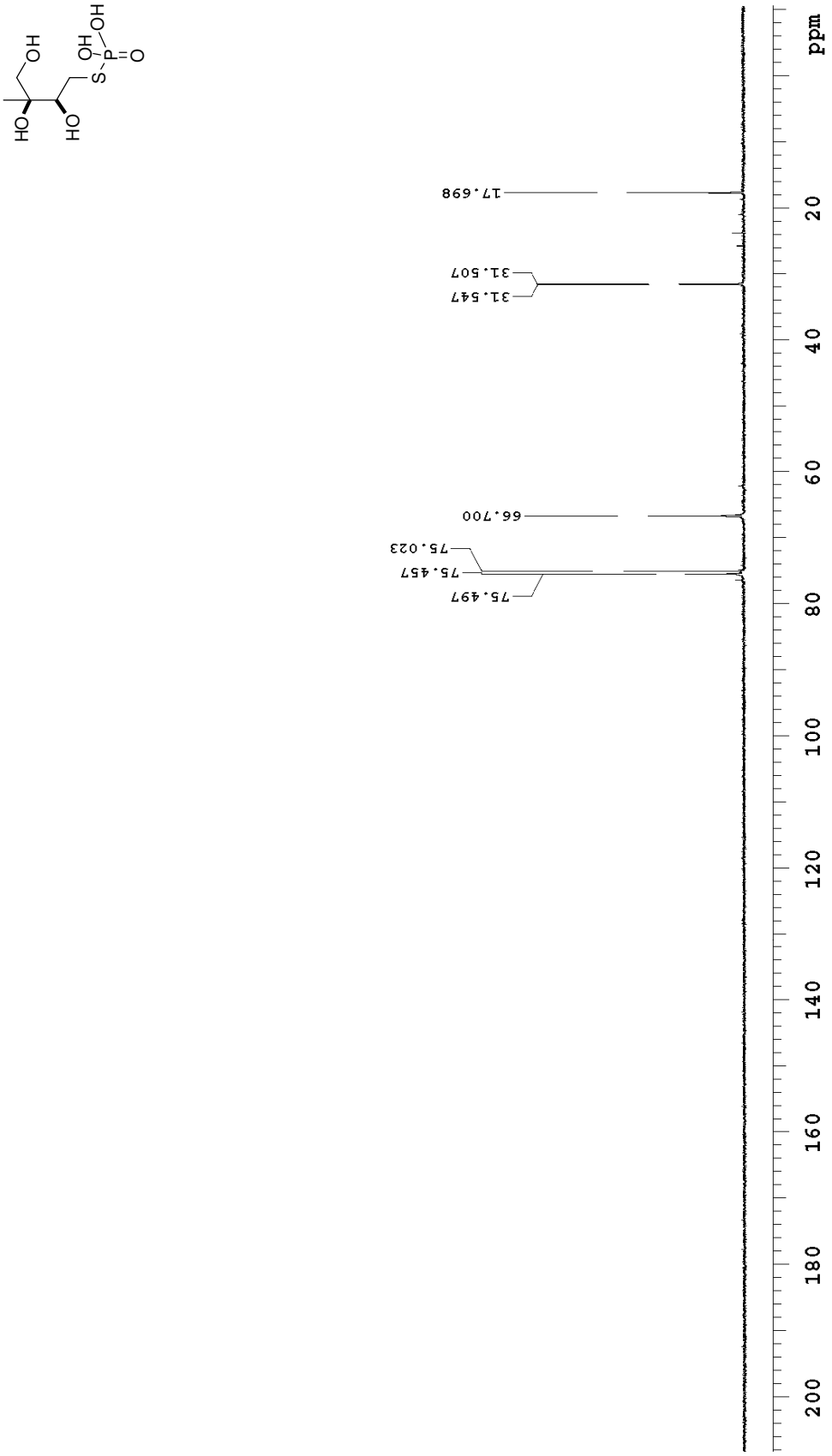

OTBDMS
HO
O
0.000
0.083
0.908
1.184
2.359
3.523
3.594
2.01
0.98
0.90
0.91.00
9.03
3.02
5.76
2.725
2.738
2.741
2.755
2.815
2.825
2.832
2.842
3.003
3.012
3.016
3.026
3.490
3.523
3.594
3.626
ppm

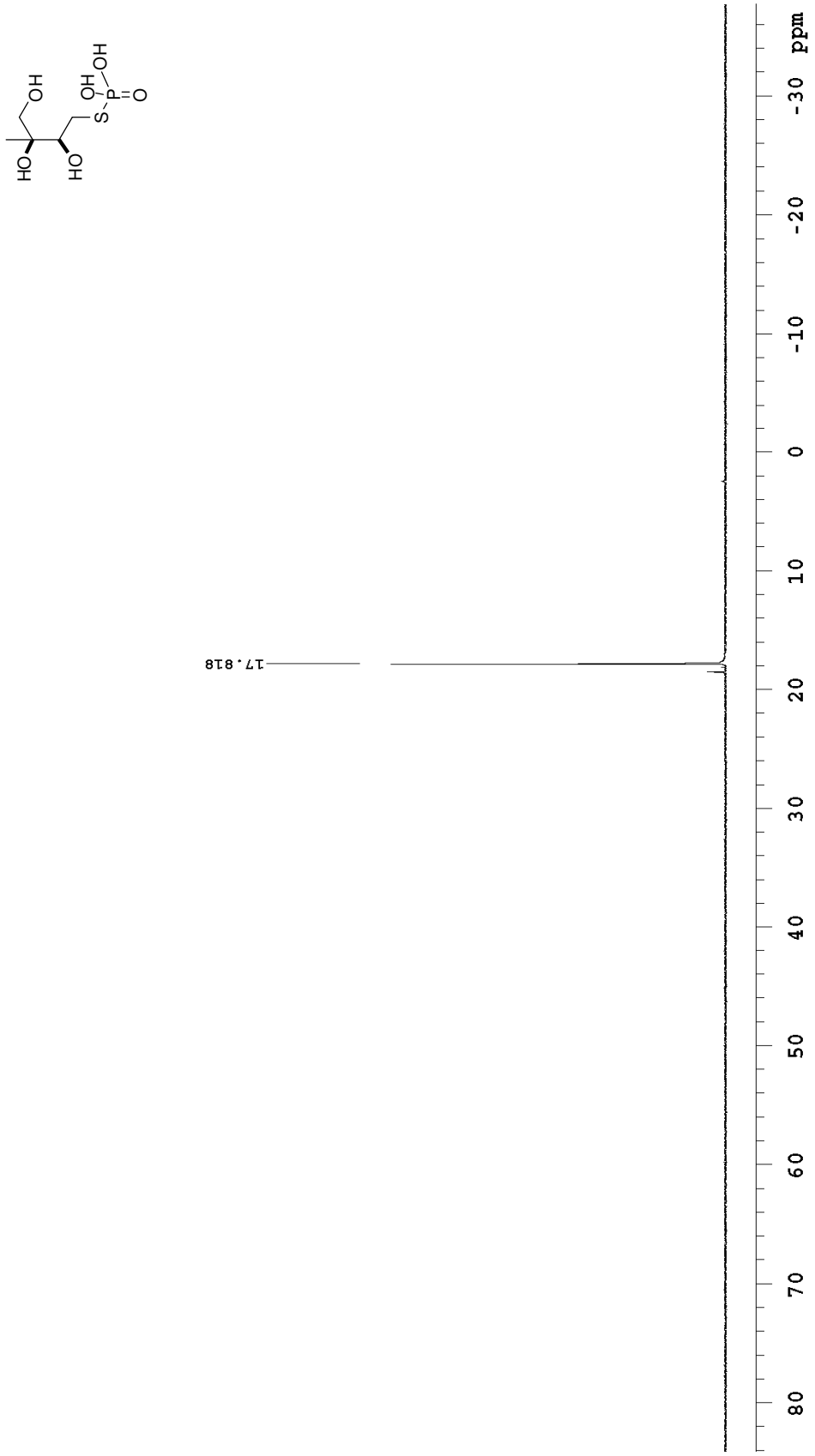

OTBDMS
HO
O
-5.561
-0.035
18.199
20.923
25.775
44.036
55.682
68.417
70.226
76.580
77.000
77.427
0 ppm
20
40
60
80
100
120
140
160
180
200


HO
OH
O
0.000
1.258
2.08
1.98
0.92
0.91
0.92
3.10
ppm
3.578
3.558
3.551
3.530
3.052
3.042
3.039
3.029
2.879
2.869
2.864
2.854
2.834
2.821
2.819
2.805
2.503
2.378
2.357
2.336
ppm


-0.041
22.284
44.489
56.550
67.209
70.292
76.573
77.000
77.420
0 ppm
20
40
60
80
100
120
140
160
180
200
OH
HO
O


4.800
3.710
3.582
3.543
3.486
3.446
3.042
3.004
2.964
2.784
2.749
2.703
2.668
3.6 3.3 3.0 ppm
3.543
3.486
1.094
0.92 0.94 0.95
1.91
3.05
8 7 6 5 4 3 2 1 0 -1 -2 -3 -4 -5 ppm
HO
OH
HO
OH
S
P
OH
O


HO
HO
OH
S
OH
P
O
OH
17.698
31.507
31.547
66.700
75.023
75.457
75.497
ppm
200
180
160
140
120
100
80
60
40
20


17.818
ppm
80
70
60
50
40
30
20
10
0
-10
-20
-30
OH
OH
OH
OH
S
P
O